2024-07-14

The Effect of Controlled Ovarian Stimulation on Kidney Function

Kharbanda, Sakshi

UNIVERSITY OF CALGARY

The Effect of Controlled Ovarian Stimulation on Kidney Function

by

Sakshi Kharbanda

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN MEDICAL SCIENCE

CALGARY, ALBERTA

JULY, 2024

ABSTRACT

Background: The global prevalence of chronic kidney disease (CKD) is >12% and prevention of CKD is a patient-identified research priority. Exogenous hormones are an important sex-specific risk factor for kidney health in females, however, it is yet unknown how the use of exogenous hormones in controlled ovarian stimulation (COS), commonly utilized in fertility treatment, effects kidney function. This study examined the effect of COS on kidney function outcomes, including measured glomerular filtration rate (mGFR) and albuminuria. **Methods:** Healthy females planning treatment with COS were recruited from the Regional Fertility Program in Calgary, Canada. Participants were studied immediately prior to initiation of COS treatment, as well as at the peak of COS. On each study day, iohexol was administered to the study participant and subsequent bloods draws were collected at time 60, 120, 150, 180, 210 and 240 minutes. Serum analysis for measurement of iohexol levels for each blood sample was used to calculate the mGFR. Albuminuria was measured via an albumin-creatinine ratio (ACR) in a spot urine sample. Changes in each outcome were assessed with Wilcoxon signed-rank tests. **Results:** Ten females initiating COS were recruited, with a median(IQR) age of 35(3) years. Most participants were nulliparous (70%), and no participants had a history of diabetes, hypertension, or CKD. Participants reported a wide variety of causes of infertility and 30% had been previously treated with COS treatment. No statistically significant changes in mGFR (p=0.13) and ACR (p=0.37) were identified during COS, though a trend towards an increase in mGFR and a decrease in ACR was demonstrated. **Conclusion:** Overall, treatment with COS did not result in any changes to kidney function, however this study is likely underpowered. Future studies with larger sample sizes are necessary to elucidate the effect of COS on kidney function, in order to optimize precision care in fertility treatment.

PREFACE

This thesis is original, unpublished, independent work by the author, S. Kharbanda.

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisor, Dr. Sandra Dumanski, whose ongoing support and mentorship have inspired my passion for advocating for women's reproductive health. Thank you for providing me with the opportunity to complete my master's degree and fostering an environment that allowed me to become a leader in the science. Also, I would like to thank my supervisory committee members, Dr. Sofia Ahmed and Dr. Shu Foong, for sharing their insightful knowledge that has been fundamental in developing the research I have pursued and will continue to. Dr. Foong has been instrumental in building a relationship between the ReACH Initiative and the Regional Fertility Program and Dr. Ahmed is a champion in sex and gender research in kidney health. Lastly, I would like to express my gratitude to Dr. Lavanya Bathini, who has graciously agreed to sit on my examining committee.

My research would not have been possible without the guidance from the lived experiences of our patient partners, study participants, nurses, and staff at the Regional Fertility Program in Calgary, Alberta. I am grateful for the time you all invested and dedicated to improving the future of fertility therapy. I also want to thank various organizations that have supported me by providing funding throughout my graduate degree, including the Canada Graduate Scholarship Master's Award by the Canadian Institutes of Health Research and the O'Brien Institute Director's Graduate Scholarship by the O'Brien Institute of Public Health. I also want to thank organizations that provided us with grants in support of this research endeavor, including the Canadian Fertility and Andrology Society for the SEED Grant and the University of Calgary for the Clinical Research Fund.

My heartfelt thanks to Victoria Riehl-Tonn, Sarah Blayney, and Mrs. Darlene Sola for helping me collect the data for my research. Also, I would like to thank my fellow lab mates who made my experience as a graduate student memorable. Finally, I am incredibly grateful to my family, friends, and loved ones for their unwavering support in conquering the challenges and celebrating the triumphs in the past two years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES & ILLUSTRATIONS

ABBREVIATIONS

Albumin Creatinine Ratio	ACR
Anti-Müllerian Hormone	AMH
Assisted Reproductive Technology	ART
Body Mass Index	BMI
Chronic Kidney Disease	CKD
Combined Oral Contraceptive	COC
Controlled Ovarian Stimulation	COS
Distal Convoluted Tubule	DCT
estimated Glomerular Filtration Rate	eGFR
Follicle Stimulating Hormone	FSH
Gender-Affirming Hormone Therapy	GAHT
Gonadotropin-releasing Hormone	GnRH
human Chorionic Gonadotropin	hCG
Intracytoplasmic Sperm Injection	ICSI
In-Vitro Fertilization	IVF
Kidney Failure	KF
Kidney Replacement Therapy	KRT
Luteinizing Hormone	LH
measured Glomerular Filtration Rate	mGFR
Ovarian Hyperstimulation Syndrome	OHSS
Proximal Convoluted Tubule	PCT
Kidney Blood Flow	KBF
Kidney Plasma Flow	KPF

CHAPTER ONE: BACKGROUND

1.1 Chronic Kidney Disease (CKD)

1.1.1 Definition and Classification of CKD

Chronic kidney disease (CKD) is defined as structural kidney damage and/or reduced kidney function for a period greater than 3 months, with implications for health . Kidney damage can be identified through pathological abnormalities demonstrated on imaging studies or kidney biopsy, abnormalities in urinary sediment, or increased urinary albumin excretion [1]. CKD is classified through a glomerular filtration rate (GFR) category and an albuminuria category and its severity manifests in 5 GFR stages and 3 albuminuria stages, as described in Figure 1 (commonly known as the kidney heat map). The severity of CKD progresses from stages G1 through G5, representing a decrease in GFR. Additionally, the severity of CKD progresses from stages A1 through A3, representing an increase in albuminuria (measured by albumin-creatinine- ratio (ACR)). As demonstrated by the kidney heat map (Figure 1), as the GFR decreases and ACR increases, the risk of kidney failure increases. The colour green represents low risk of progression of kidney disease, and as the GFR and ACR progress, the risk increases to yellow (moderate risk), orange (high risk) and red (very high risk) [2]. The end-stage of the progression of CKD, known as kidney failure (i.e. category G5 CKD), can be treated with kidney replacement therapy (KRT) in the form of dialysis or kidney transplantation [3].

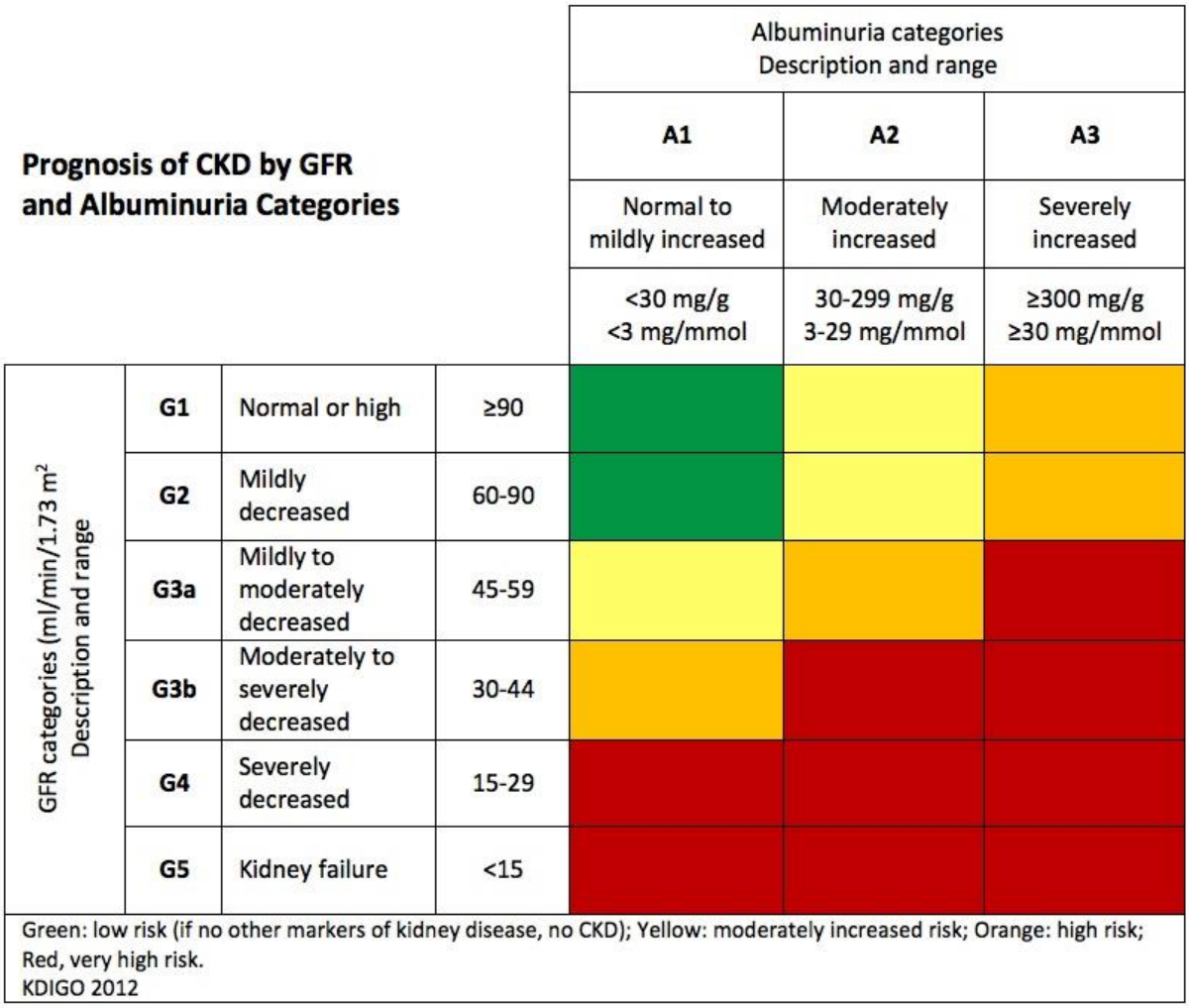

Figure 1. Kidney heat map outlining the classifications of CKD associated with GFR and albuminuria.

1.1.2 Prevalence & Impact of CKD

The global burden of CKD is substantial, and the prevalence of CKD is approximately 11-13% [4] and rapidly increasing [5, 6]. CKD is one of the top 10 causes of death worldwide, and its impact on global mortality has been increasing over time [7-9]. By 2040, CKD is predicted to become the 5th leading cause of death worldwide [10] and a 41.5% increase in global all-cause mortality rate was associated with CKD between 1990 – 2017 [11]. In Canada, the prevalence of advanced CKD

(classification G3-G5 only) was recently reported to be 71.9 per 1000 individuals in a study using data from primary care services [12]. In fact, the annual cost for treatment for advanced CKD exceeds $40 billion per year in Canada resulting in an extensive financial burden on the healthcare system [13, 14]. The burden of living with CKD extends beyond financial costs, and CKD grossly impacts individuals' quality of life negatively as well [15, 16]. Thus, prevention of CKD has been established as one of ten primary research priorities by individuals living with CKD [17].

1.1.3 Sex and/or Gender Differences in CKD

The prevalence of CKD in females is ~12% and in males is ~10%, demonstrating that females are at higher risk for developing CKD compared to males [18]. However, compared to men, there are less women on kidney replacement therapy (KRT) and women tend to start KRT later in life, which may suggest a reduced risk of progression of CKD in women [19-22]. Additionally, females with CKD report a higher symptom burden and symptom severity compared to males [23]. Importantly, previous literature has also demonstrated that there is a steeper cardiovascular mortality curve in females compared to males when assessing the risk association of reduced eGFR and albuminuria. Thus, the mortality risk associated with CKD in females is significant [24]. Although, sex and gender are often used interchangeably used in literature, it is has become clear that both factors likely influence kidney health and disease [25].

1.1.4 Reproductive Implications of CKD

CKD is associated with complications in female and male reproductive health [26-29] . Although the complications manifest differently between sexes [30], common reproductive abnormalities in individuals living with CKD include disruption of sex hormone balance, gonadal dysfunction and

sexual dysfunction. In females, this can manifest as abnormal menstruation and ovarian function, impaired sexual health, and reduced fertility [26]. As an individual progresses through the stages of CKD, the reproductive abnormalities caused by imbalance in sex hormones appear to increase in severity [31]. However, individuals living with CKD may begin experiencing reproductive health dysfunction even in the early stages of CKD [28, 32-34]. Impaired fertility and reduced reproductive function are one of the most important implications of CKD, yet it is an often underrecognized complication in individuals living with CKD [35, 36]. Based on data from kidney registries, the pregnancy rate among individuals treated with kidney transplant and individuals treated with dialysis compared to the general population are 10% and 1%, respectively [37]. Thus, these data highlight the importance of reproductive health in individuals with CKD.

A small number of studies have examined the impact of CKD, and especially kidney failure (KF), on reproductive hormones in females with CKD [31]. The disruption in hormonal balance varies across the different stages of CKD and maybe influenced by comorbidities as well as associated treatments making it challenging to determine sole causation [28, 38, 39]. In females with CKD, the pulsatile release of GnRH may be hindered resulting in the absence of the cyclic release of LH and FSH, but LH levels remain high [40-42]. As a result, estrogen levels may be reduced due to the lack of cyclicity which causes a disruption in the feedback mechanism of estrogen in relation to the hypothalamus and anterior pituitary [43]. The consistent decreased level of estrogen, increased level of gonadotropins and loss of cyclicity in the release of sex hormones may result in anovulation [28]. Additionally, prolactin levels may be increased as well due to reduced kidney clearance and increased production related to reduced sensitivity to dopaminergic inhibition [44] (Figure 2). Prolactin is a critical hormone in fertility as it is a natural suppressant of ovulation, a

key component of successful reproduction [45, 46]. Interestingly, despite the disruption in sex hormone balance in females with CKD, studies have found that the abnormalities may be reversible with kidney transplantation or increased dialysis dosing [28, 47, 48]. Additionally, Anti-Müllerian Hormone (AMH), a key marker of ovarian reserve, is reduced in females with CKD compared to the general population, beginning in the early stages of CKD [34, 49]. There are conflicting results on the AMH levels in females living with KF treated with dialysis, some studies found an increased level of AMH in this population potentially due to reduced kidney clearance [50, 51]. On the contrary, recent studies reported that there was a decrease in AMH levels in females treated with dialysis [34, 49].

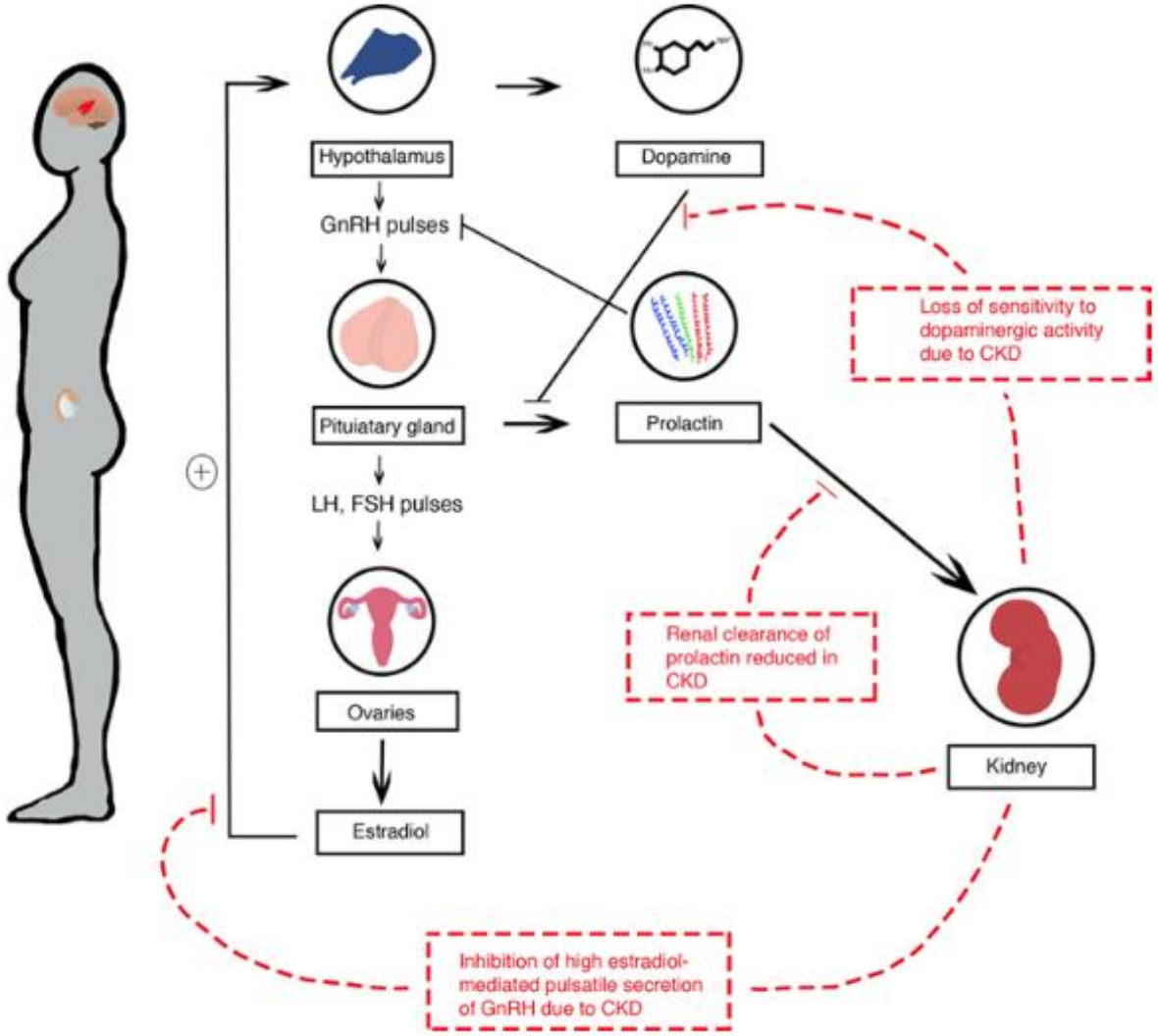

Figure 2. Disruption of the hypothalamic-pituitary-ovarian axis in females with CKD. Reprinted with permission from Dumanski et al.

1.2 Kidney Health and Function

1.2.1 Kidney Anatomy

The kidneys are two bean-shaped organs that are located between the abdominal organs and muscles of the back [52, 53]. The kidney comprises of the cortex and the medulla. The cortex includes the glomeruli, and proximal/distal convoluted tubules, as well as the cortical collecting

ducts, and vasculature [54]. The medulla consists of the vasa recta, a network of capillaries forming the countercurrent exchange system, the loop of Henle, as well as the collecting tubules [55]. These tubules have several ion and water channels as well as transporters that are responsible for adjusting the concentration and composition of the urinary filtrate [55]. The kidney consists of approximately 2 million nephrons [56], the functional unit of kidney, and each nephron comprises of a glomerulus with capillaries responsible for blood filtration[57] . The nephron also consists of a complex tubular system alongside the glomerulus, including the proximal convoluted tubule (PCT) in the renal cortex, the loop of Henle, a hairpin-like structure that penetrates the medulla, and the distal convoluted tubule (DCT) [58, 59]. The nephron eventually releases filtered waste and water into the urinary system for excretion from the collecting duct [60].

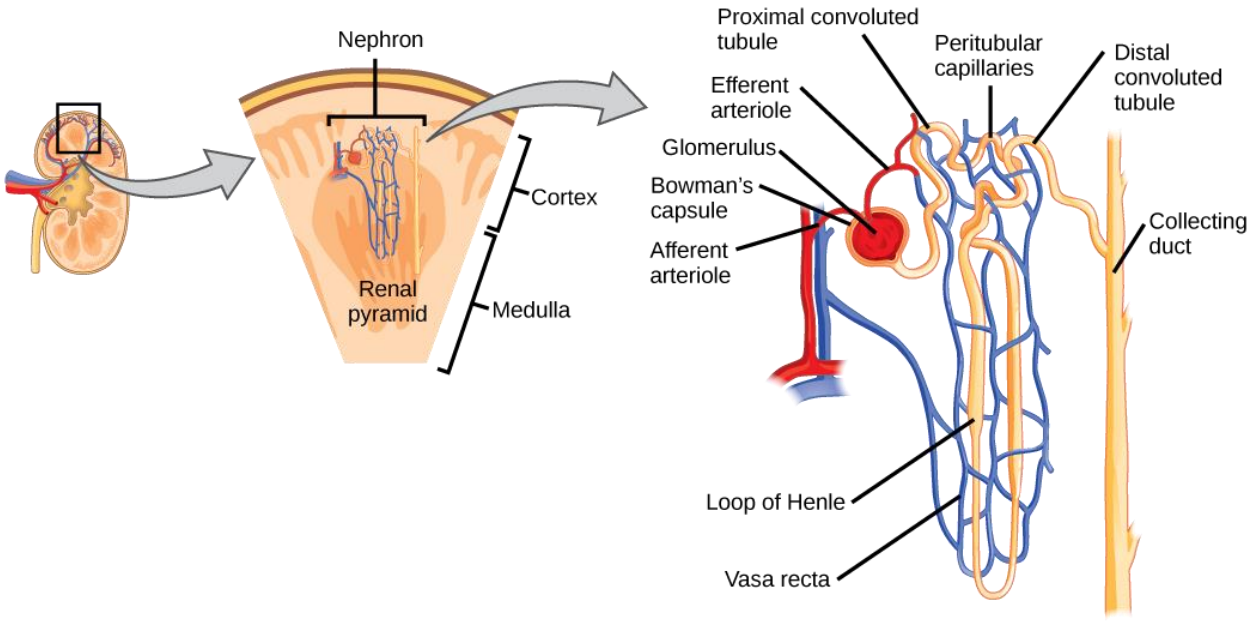

Figure 3. Kidney and Nephron Anatomy [61].

1.2.2 Kidney Physiology

The kidneys receive 1.0 to 1.1 liters per minute of blood from the cardiovascular circulation, known as the kidney blood flow (KBF), some of which is directed into each glomerulus within the kidney's nephrons [62]. However, only plasma can pass through the capillary beds within the glomerulus, and this portion of the KBF is classified as the kidney plasma flow (KPF) (approximately 600-720 mL per minute) [62]. From the KPF flowing through the glomeruli, solutes and water are filtered into Bowman's space, an enclosed space within the Bowman's capsule which represents the beginning of the urinary space and is known as glomerular filtration [53, 63]. The filtration membrane within each glomerulus is a uniquely designed barrier that is selective in nature, preventing the passage of substances based on their size and charge [53]. It consists of three layers including the fenestrated endothelium which only allows plasma and plasma-based components to pass through, the basement membrane which is a negatively charged barrier that prevents proteins from entering Bowman's space (such as albumin), and podocytes which consist of foot processes that enhance selective filtration [64]. Through Bowman's space, the filtered solutes and water enter the complex tubular system of the nephron [63]. There are four segments within the tubular system- the PCT, the loop of Henle, the DCT, and the collecting tubule [58, 59]. In the tubule, solutes and water are reabsorbed into the blood system through a complicated process involving active and passive transport [65]. Once the filtrate has been altered through tubular secretion and absorption in the nephron's tubular system, it will become urine for excretion [66].

1.2.3 Measurement of Kidney Function

1.2.3.1 Measurement of Glomerular Filtration Rate (GFR)

The glomerular filtration rate (GFR), most commonly measured in milliliters per minute, is the primary measure of kidney function and is defined as the rate of blood plasma filtration from the capillaries in the glomerulus into Bowman's space [62]. An individual's GFR is adjusted for body surface area to account for differences in kidney size and is based on $1.73m^2$ surface area [67]. A normal GFR in healthy adults is approximately 120 mL/min/$1.73m^2$, though a GFR above 90 mL/min/$1.73m^2$ is often considered healthy [2]. To measure GFR, the rate of clearance of an ideal filtration marker is estimated through blood or urine sampling, and estimation using inulin is the gold standard method [68]. The characteristics of an ideal filtration marker include 1) clearance that is not reliant on extrarenal routes, and 2) the capability of the substance to be freely filtered by the glomerulus and excreted in the urine in the absence of tubular reabsorption or secretion [69]. However, clearance of inulin is impractical because it not only requires a continuous intravenous infusion and multiple, timed urine collections; it also requires a complicated chemical assay that is expensive and not readily available [68]. Some alternatives to inulin are isotopic substances (i.e., ^{125}I-iothalamate or ^{51}Cr-EDTA) or non-isotopic substances (i.e., iohexol) that can also be utilized to measure GFR [70]. A less burdensome method for estimating GFR utilizes mathematical equations to determine estimated GFR (eGFR) through serum concentrations of endogenous filtration markers such as creatinine and/or cystatin C (e.g. Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) equations)[71-73]. Although, using an eGFR equation is fast, cost-efficient, and widely used in clinical practice [74], measured GFR (mGFR) can be beneficial to determine true kidney function, as well as how it relates to kidney blood flow [71]. Assessment of accurate kidney function is especially important during clinical decision-making when considering important clinical decisions such as kidney donation, dosing of medication and more [75].

1.2.3.2 Iohexol-based measurement of GFR

The use of iohexol in humans was first reported in a study published in 1980 [76]. It was injected in healthy volunteers in increasing doses (125–500 mg I/kg) and they found that the substance was safe and fully excreted by kidneys [76]. Since the 1980s, iohexol has been extensively assessed for its safety [77, 78]. It also possesses many traits that are in line with an ideal filtration marker such as its ability to almost entirely be excreted through glomerular filtration without being reabsorbed, secreted or metabolized by the kidney [79]. It is shown to be consistent with the gold standard inulin clearance and is now recommended as an appropriate standard for evaluating kidney function [70, 80-82]. In one study comparing methods, 20 healthy individuals received bolus doses of inulin and iohexol to assess for plasma clearance, as well as continuous infusions of inulin and iohexol to assess for urinary clearance. The results of this study demonstrated that plasma and urinary clearance of both substances were similar (ie similar mGFR was resultant from all four methods) and that iohexol is an appropriate marker for mGFR in healthy individuals [83].

1.2.3.3 Measurement of Albuminuria

Albuminuria is one of the most important and widely used biomarkers of chronic kidney disease [84]. Albumin is the most common plasma protein and normally excreted in very low amounts in the urine [85]. The structure of the glomerulus determines the size and charge of the substances able to pass through its membrane [62], and given its high molecular weight (69 kDa), albumin cannot easily pass through the glomerular membrane [85]. Thus, when albumin is detected in the urine in increasing amounts, it represents impaired glomerular function [86]. Historically, the standard for measuring the amount of albumin excreted into urine is through collection of urine in a 24-hour period [87]. However, 24-hour urine collection is poorly feasible due to the cumbersome

nature of collection, as well as storage and accuracy in timing [88]. Alternatively, it is now well accepted that the urine albumin concentration can be indexed to urine creatinine concentration and is reported as urinary albumin-to-creatinine ratio (ACR) [89]. Further, the ACR has a similar diagnostic performance as a 24-hour urine albumin excretion rate [2, 90].

1.2.3.4 Measurement of Albuminuria by ACR

To measure ACR, a spot urine sample is used to quantify albuminuria. In this test, the measured urine albumin level is adjusted according to the urine creatinine level to account for differences in urine concentration or dilution [91]. To quantify the urinary albumin and creatinine, immunoturbidimetric assays can be used. In this method, the anti-albumin antibodies from each reagent bind to the antigens in the sample material and create an antigen-antibody complex which are further measured turbidimetrically [92]. Many studies have assessed the correlation between spot urine ACR and the 24-hour urine collection for albuminuria, including in populations who have preeclampsia, IgA nephropathy, chronic kidney disease and others [93-96]. Each of these demonstrated that the ACR can be used as a surrogate measure for measuring albuminuria [93-96]. Equation 1 can be used to calculate the ACR [97].

$$uACR = \frac{urine\ albumin\ (mg/L)}{urine\ creatinine\ (mmol/L)}$$

Equation 1. Equation for urinary albumin-to-creatinine ratio

1.3 Reproductive Hormones and Kidney Function in Females

1.3.1 Endogenous Sex Hormones & Kidney Health

1.3.1.1 Estrogen

Studies have shown that endogenous estrogen may have nephroprotective properties in animal models and humans [98-102]. Estradiol and estriol have been associated with reduced albuminuria and other mediatory systems of glomerular damage in rat models [98, 99]. A small amount of literature suggests that kidney function in females with CKD declines slower than in males with CKD and that this difference may not be observed after menopause, suggesting nephroprotective properties of estrogen [100-102]. One study found that premenopausal women who underwent bilateral oophorectomy were at higher risk of developing CKD, further suggesting a protective effect of estrogen [103]. Further, dynamic changes in kidney physiology have been demonstrated throughout the menstrual cycle, with increased GFR and KPF in the high-estradiol luteal phase, indicating a potential role for estrogen [104]. In a 15-year prospective cohort study, scientists assessed the effect of endogenous estrogen exposure on CKD and found that a lower duration of endogenous estrogen exposure among reproductive age women is a risk factor for CKD [105]. Although, the specific impact of endogenous estrogen on kidney function remains unknown, it appears to provide nephroprotective benefits.

1.3.1.2 FSH

Other studies have investigated the effects of follicle-stimulating hormone (FSH) on female kidney health [106, 107]. In one study, they examined the blood samples from 3055 postmenopausal women and found that there was a strong, independent negative correlation between eGFR and

FSH levels [106]. They adjusted for confounding factors such as age, current smoker status, body mass index (BMI), total testosterone, estradiol and LH and others to further assess the relationship; and found that there was a three-fold increase in FSH in the highest odds risk for decreased eGFR group [106]. Additionally, a cross-sectional study including 624 pre-menopausal, 121 peri-menopausal and 2540 post-menopausal women examined the effect of decline in kidney function on FSH levels [107]. The study demonstrated that eGFR declined from premenopause to perimenopause to menopause, and that in the menopause group, the odds of CKD increased with higher serum FSH levels [107].

1.3.1.3 Other endogenous sex hormones

There are not many studies assessing the relationship between other endogenous hormones, such as luteinizing hormone (LH), progesterone, prolactin, and gonadotropin-releasing hormone (GnRH), and kidney health. A study was conducted to assess the relationship between LH and diabetic kidney disease in individuals living with type 2 diabetes mellitus. This study observed that in postmenopausal women that were not taking hormone therapy or hormonal contraceptives, higher levels of LH demonstrated higher odds of macroalbuminuria compared to those with the lowest levels of LH [108].

1.3.2 Exogenous Sex Hormones & Kidney Health

1.3.2.1 Combined Oral Contraceptive (COC) & Post-Menopausal Hormone Therapy (PHT)

The combined oral contraceptive (COC) is one of the most widely prescribed medications worldwide [109]. Currently, 151 million women of reproductive age use the oral contraceptive pill [109]. It is commonly used to prevent pregnancy and/or to treat abnormal menstruation,

premenstrual syndrome, perimenopausal vasomotor symptoms, and acne or hirsutism [110]. The COC consists of estrogen and progesterone [111]. Similarly, post-menopausal hormone therapy (PHT) is comprised of estrogen (with or without progesterone) and is commonly prescribed to treat symptoms of perimenopause and prevent chronic conditions such as osteoporosis in women [112].

The hormones administered through COC and PHT may have significant implications on female health [113]. Most studies have shown that there is a positive association between oral contraceptive use and urinary albumin excretion [114-117]. One study demonstrated that women using oral contraceptives and post-menopausal hormone therapy had an increased odds ratio for albuminuria of 1.9 and 2.05, respectively, compared to non-users, [116]. Another study found a significant reduction in albuminuria among women that stopped using oral contraceptives suggesting a reversible effect if oral contraceptives were discontinued [118].

It remains unclear how COC and PHT impact GFR in users. The studies to date demonstrate mixed results, though a systematic review and meta-analysis of studies addressing the effect on OCP and kidney health outcomes, including GFR, revealed no obvious effect [119]. In a study assessing the impact of oral estrogen therapy in postmenopausal women, it was found that PHT use was associated with a significant reduction in GFR compared to those who were not on postmenopausal hormone therapy [120]. Additionally, this study accounted for the route of administration and showed that the increased rate of decline in kidney function was associated with oral but not transvaginal estrogen use [120]. Moreover, it showed that estrogen has a dose-dependent association with reduction in kidney function in postmenopausal women [120]. Conversely,

another study assessed the effect of PHT on 85 postmenopausal women [121]. This study found that individuals who were using PHT for 30 weeks had a significant increase in their GFR. Thus, there was a positive association between PHT and GFR [121]. Exogenous progesterone administration has also been independently associated with an increase in plasma volume [122, 123], RAAS activity, and the RAAS-associated kidney blood flow [124], likely having implications for kidney health and function.

1.3.2.2 Gender-Affirming Hormone Therapy

Gender-affirming hormone therapy (GAHT) may be prescribed to individuals whose gender identity does not align with their sex assigned at birth [125, 126]. It alters the biochemical hormonal balance to reflect one's affirmed gender identity and is also known to influence the body composition, including lean muscle mass and body fat [127, 128]. For individuals who were assigned female sex at birth that want to transition to their gender identity as a man, GAHT includes testosterone therapy delivered via injection or transdermal method [126]. Although, there are limited studies assessing the impact of GAHT on kidney function in the transgender population, one study examined the 24-hour urine creatinine excretion in transgender populations and found that there was a significant increase in urine creatinine excretion in transgender men treated with testosterone [129]. Moreover, a systematic review assessing the effect of GAHT on measures of kidney function found that after initiation of GAHT for 12 months, the serum creatinine levels increased in transgender men [130]. Considering that eGFR equations consist of sex as a coefficient to account for muscle mass differences in cisgender men and women, there may be discrepancy in the calculated eGFR for transgender populations who are using GAHT [131].

Additionally, the use of eGFR equations in transgender individuals on GAHT has not been validated [132].

1.4 Infertility & Fertility Preservation

1.4.1 Infertility

1.4.1.1 Definition & Types of Infertility

Infertility is formally defined as the inability to achieve pregnancy after 12 months or more of regular, unprotected sexual intercourse [133]. Infertility can be characterized into two broad categories: biological and social infertility [134]. Biological fertility is specific to heterosexual relationships, and may be classified as male-factor, female-factor, or unexplained infertility. Female- factor infertility may be resultant from polycystic ovary syndrome (PCOS), endometriosis, ovulatory disorders, tubal abnormalities, and others [135]. Comparatively, male-factor infertility may be caused by abnormal spermatogenesis, reproductive tract anomalies, or inadequate sexual and ejaculatory functions [136]. Unexplained fertility can also occur in heterosexual couples trying to conceive [137]. Conversely, social infertility describes infertility related to sexual orientation or relationship status, and may include individuals from the Two-Spirit, Lesbian, Gay, Bisexual, Transgender, Queer, Intersex+ (2SLGBTQI+) community [134].

1.4.1.2 Prevalence & Impact of Infertility

Infertility is estimated to affect 10 – 25 % of reproductive-aged couples globally, highlighting the urgency for safe and feasible fertility treatments [138]. There are negative consequences of infertility within the psychosocial wellbeing of an individual such as martial instability, social

isolation and stigmatization [139-142]. Additionally, there are challenges faced by individuals experiencing infertility including lack of access to fertility treatment [143] and limited to no coverage by insurance and government funding for fertility treatment [143, 144]. In terms of direct costs associated with assisted reproductive technology (ART), one study found that it ranged from as low as $24,373 (USD) to as high as $38,015 (USD)[145]. Additionally, the percentage of annual income utilized to expense the cost of in-vitro fertilization (IVF) treatment after receiving government funding also varied between countries ranging from 6% in Australia to 50% in the United States [146]. Thus, studies that assess the economic burden of ART-related treatments on individual and the healthcare system suggest that affordability is one of the primary factors that influence the ability to access treatment and choice of treatment.

1.4.2 Treatment for Infertility

1.4.2.1 Assisted Reproductive Technology (ART)

1.4.2.1.1 Definition of ART and Types of Fertility Treatments

Although there are multiple options individuals with infertility to have a family, such as adoption or surrogacy, medical treatment may also be effective[147, 148]. Treatment for infertility may utilize a variety of fertility treatment options, including assisted reproductive technology (ART) [149]. ART is defined as fertility treatments in which oocytes or embryos are manipulated in a laboratory [149]. For example, ART includes in-vitro fertilization (IVF) treatment, in which fertilization of human oocyte occurs outside of the body, the embryo(s) is developed in a laboratory, and then transferred to the uterus [150]. Non-ART fertility treatments include administration of fertility drugs that induce or enhance ovulation in addition to timed intercourse or use of intrauterine insemination (semen deposited into female upper uterine cavity)[151, 152].

1.4.2.1.2 In Vitro Fertilization (IVF)

In-vitro fertilization (IVF) is a form of assisted reproductive technology (ART) developed to treat infertility [150]. In Canada, greater than 35,000 IVF treatment cycles are performed each year in 36 specialized clinics [153]. IVF is currently responsible for approximately 2% live births in Canada and its use is rapidly rising [153-155].

1.4.2.1.3 Steps in IVF Treatment

IVF treatment consists of 4 primary steps (Figure 4):

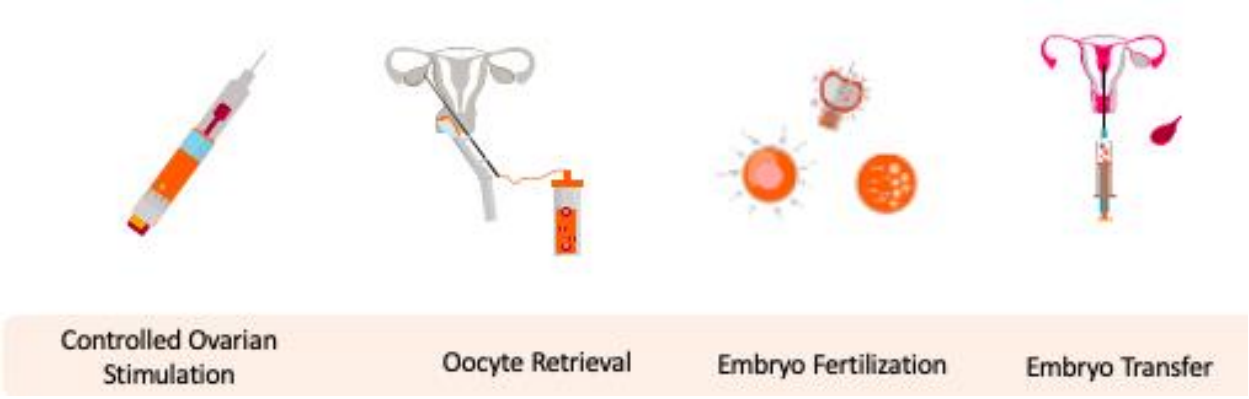

Figure 4. Steps of In Vitro Fertilization Treatment

1) Controlled Ovarian Stimulation:

The first step of IVF involves controlled ovarian stimulation, typically employing administration of exogenous gonadotropins (FSH +/- LH)[156]. COS, and specifically gonadotropin treatment, results in the development of multiple ovarian follicles [157]. Concurrently, gonadotropin-releasing hormone (GnRH) agonists or antagonists are used to suppress the natural LH release to allow continued follicle growth during COS [158].

2) Oocyte retrieval:

Mature oocytes are retrieved 34-36 hours following maturation, which is typically induced with exogenous hCG administration [156]. Transvaginal ultrasound is utilized to guide a needle to aspirate the oocyte from each accessible follicle [159].

3) Embryo Fertilization:

The oocytes are fertilized by incubating the oocytes with sperm or by intracytoplasmic sperm injection (ICSI) [156]. The ejaculated semen contains sperm that are isolated via density centrifugation and washed in a media of high protein concentration [156]. To encourage fertilization, the sperm are incubated with the oocyte for 12-18 hours. Comparatively, in ICSI, an immobilized sperm is injected individually into an oocyte. This technique can be useful in cases where male-factor infertility related to sperm quality plays a role, because it allows the sperm directly enter the oocyte without the need to penetrate the zona pellucida [156].

4) Embryo Transfer:

After fertilization occurs, the embryo(s) are incubated until ready for transfer. Most commonly, embryos may be transferred into the uterus at two stages: either on day 3 (embryo cleavage stage) or on day 5 (embryo blastocyst stage) after fertilization [159]. A catheter is passed through the cervix, guided by a transabdominal ultrasound, and the embryo(s) is placed in the uterus from the catheter. To ensure the uterus is prepared for embryo implantation, progesterone is provided to the female undergoing IVF following oocyte retrieval [156].

1.4.4 Fertility Preservation

1.4.4.1 Oocyte & Embryo Cryopreservation

Fertility preservation allows individuals to preserve gametes or embryos for use at a future time point [160]. Fertility preservation may be medically warranted in individuals who may undergo gonadotoxic treatment due to health conditions such as cancer, lupus and others [161]. It may also be important for those who may want to delay child-bearing (i.e., age related fertility preservation) [162]. Similar to in-vitro fertilization, the process for fertility preservation in females includes COS and oocyte retrieval [163]. Following oocyte retrieval, cryopreservation is utilized to preserve healthy oocytes, or may be utilized following embryo fertilization to preserve healthy embryos [161, 163-165]. To undergo cryopreservation, the oocytes or embryos are stored in -196 °C of liquid nitrogen and at these low temperatures, water exists in solid state and no biological reactions take place [166]. When it is time to utilize these mature oocytes or embryos, they are brought back to a temperature that supports viability at 37 °C, though not all oocytes or embryos survive the defrosting procedure [167].

1.5 Controlled Ovarian Stimulation (COS)

1.5.1 Exogenous Hormones in COS

Clinicians commonly tailor the details of the COS treatment (for IVF or fertility preservation) based on patient age and markers of ovarian reserve including antral follicle count, anti-Müllerian hormone (AMH) levels, and/or FSH and estradiol levels [168, 169]. However, there are two primary COS protocols including the GnRH agonist and GnRH antagonist protocols [170, 171].

The GnRH agonist protocol is begins with the administration of daily GnRH agonist starting on day 21 of the previous cycle in the luteal phase [172]. The purpose of GnRH agonist is to suppress the release of gonadotropins from the pituitary gland during ovarian stimulation [173]. Thus, it is administered consistently throughout the entire COS cycle. Simultaneously, exogenous gonadotropins (FSH +/- LH) are also administered starting on cycle day 3 of the following menstrual cycle [156]. Throughout the process, adjustments to hormone dosages are made based on growth of follicles [174]. Finally, the hormone injection for oocyte maturation (usually hCG) is administered only when at least three follicles reach a size of 18 mm [174].

The GnRH antagonist protocol begins with the administration of daily exogenous gonadotropins (FSH +/- LH) beginning on day 3 of the menstrual cycle [174]. Administration of the GnRH antagonist begins approximately 5 days following initiation of exogenous gonadotropins and is used to block the natural LH surge that may be induced by follicle growth [174]. Finally, the hormone injection for oocyte maturation (usually hCG) is administered only when at least three follicles reach a size of 18 mm [174].

Importantly, variations of the above protocols may occur, including pre-COS treatment with combined oral contraceptive pill or estrogen [175, 176].

1.5.2 Impact of COS on Endogenous Hormones

During COS, the female's endogenous estradiol levels increase up to 20 times from baseline [177]. Exogenous follicle stimulating hormone (FSH) is administered in high doses during COS, which

results in an increased number of estrogen-secreting ovarian follicles produced by the ovaries [156] (Figure 5).

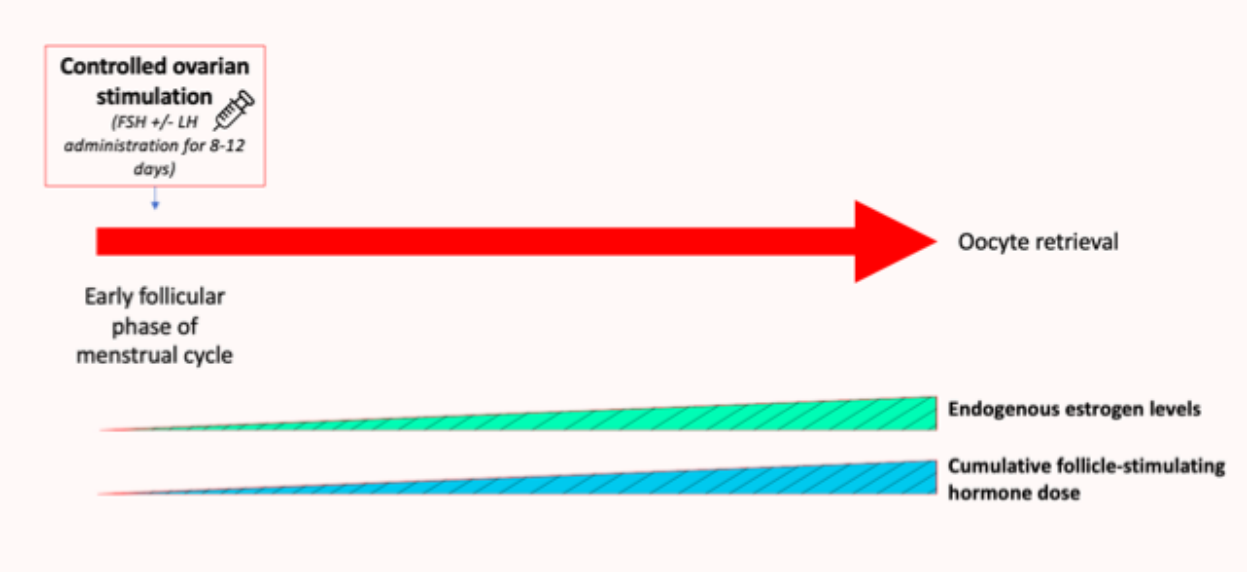

Figure 5. Visual Representation of COS as it relates to hormone doses and levels. Reprinted with permission from Elaha Niazi.

1.5.3 Risks associated with COS

ART technologies such as COS may result in complications [178]. The most severe and life threatening complication is ovarian hyperstimulation syndrome (OHSS) [156]. In OHSS, individuals experience abdominal distension, nausea, and vomiting, resulting from increased vascular permeability [179]. In severe cases of OHSS, individuals show signs of hypovolemia, elevated creatinine, electrolyte abnormalities and others [180, 181]. OHSS can result in thromboembolism, acute kidney injury, disseminated intravascular coagulation, as well as death [156]. According to the World Health Organization (WHO), the incidence of severe OHSS is between 0.2-1% all COS cycles [182]. Additionally, use of ART, including IVF, is associated with more frequent pregnancies with twins and triplets [183]. According to a study conducted to assess

live birth data between 1980 to 2015, 19% of all twins and 25% of all triplets are related to IVF [184]. This is important because multiple gestations have been associated with an increased risk for hypertensive disorders during pregnancy and preterm birth [185]. Moreover, a systematic review assessing a number of studies found that singleton IVF pregnancies are associated with increased risk of many disorders in the female and fetus including hypertensive disorders during pregnancy, gestational diabetes, antepartum hemorrhage, congenital abnormalities, cesarean sections, preterm delivery, low birth weight, small for gestational age, and perinatal mortality [186].

1.6 COS and Kidney Function

Although it is understood that both endogenous and exogenous hormones, including estrogen and FSH, impact kidney function; it is yet unknown what influence COS treatment has on kidney function.

CHAPTER TWO: STUDY RATIONALE AND HYPOTHESIS

2.1 State of the Science

1. The global prevalence of CKD in females is 12% and it is increasing with time [18].
2. Individuals living with CKD have identified prevention of CKD as one of top 10 priorities in research [17].
3. Use of various forms of exogenous hormone administration (i.e., oral contraceptives and post-menopausal therapy) have been linked to an increase in kidney risk [120, 187].
4. Use of COS in IVF and fertility preservation is rapidly increasing in Canada [154]
5. it is unknown whether exogenous hormone administration in COS treatment has an effect on kidney risk.
6. This research will provide increased knowledge on the implications of COS on kidney function, inform future studies and clinical decision-making, and empower females and their care providers in their reproductive choices.

2.2 Hypothesis and Objective

Research Question: What is the effect of COS on kidney function in females treated with IVF or fertility preservation?

Objective: To examine the effect of COS on important kidney function outcomes, including 1) mGFR and 2) ACR.

Hypothesis: COS treatment will result in an increase in both mGFR and ACR.

CHAPTER THREE: METHODS

3.1 Study Population

3.1.1 Inclusion Criteria

- Female sex assigned at birth
- Age 18+
- Planning to undergo COS treatment for fertility preservation or in-vitro fertilization treatment

3.1.2 Exclusion Criteria

- Inability to commit to study time commitments
- Inability to provide informed consent

3.1.3 Study Participant Recruitment

Participants were recruited from the Regional Fertility Program (RFP) in Calgary, Alberta. Formal recruitment education sessions on the study were provided to all RFP clinical staff. Individualized follow-up of recruitment activities following recruitment education session were offered to all clinical staff at RFP. Recruitment materials included study posters advertised in waiting rooms and examination rooms, as well as study handouts provided to patients accessing fertility assessment for ART. Furthermore, electronic study invitation letters were provided to all patients accessing ART at the time of cycle set-up. Interested individuals were asked to contact the study coordinators and individuals who were interested in the study underwent an eligibility screening process by phone with a study team member. All recruitment material, as well as recruitment

training for the RFP staff, encouraged participation from a wide spectrum of gender identity and diversity in family type. Recruitment materials exhibited gender-inclusive language and symbols.

3.2 Data Collection

3.2.1 Baseline Data Collection

Demographic Information:

A semi-structured interview was conducted, and participants provided the following information: age, ethnicity, sexual orientation, sex assigned at birth, gender identity, and gendered variables including postal code as a surrogate marker of socioeconomic status (through median neighborhood income).

Medical History:

A focused medical history was collected through a semi-structured interview and included a detailed reproductive history (including type of infertility, previous fertility treatment, and pregnancy history), medication history (including previous or current exogenous sex hormone use), and medical history (including history of established cardiovascular and kidney risk factors). A formal chart review of medical consultation notes, laboratory values and current medications was also completed.

Physical Examination:

A physical examination at each study visit included height, weight, body mass index (BMI), abdominal circumference, and blood pressure. To measure the weight, participants were instructed to stand barefoot on the center of a calibrated digital scale (with minimal clothing and after urine

sample was collected)[188]. The height was measured using a non-elastic flexible tape and participants were instructed to stand straight, barefoot against a wall or a flat surface. In order to calculate the body mass index (BMI), the weight (measured in kilograms) was divided by height-squared (in meters) [189]. For the abdominal circumference, the patient was instructed to stand straight with a bare midriff after the patient exhaled and had their feet touching each other as well as hands hanging freely [190]. Then, the midpoint between the lowest rib and just above the iliac crest was measured using a measuring tape [190]. The measuring tape was placed in a 90 degree angle made between the body and the floor and tension was applied without putting pressure on the abdominal wall [191]. Finally, the blood pressure was measured using an automated blood pressure machine per the Hypertension Canada guidelines [192]. The participant was instructed to sit upright in a relaxed position with their feet flat and legs uncrossed [192]. Then, the cuff was placed in a manner that the middle of the cuff was around the bare upper arm at the heart level and the lower edge 3 cm above elbow crease [192]. Three automated blood pressure measurements were taken; the first one was discarded, and the other two were averaged.

Laboratory Measurements:

At each study visit, a baseline blood draw was collected by inserting an 18-gauge peripheral venous cannula into the antecubital vein. To analyze the hormone levels from the blood, chemiluminescence immunoassays were used. This technique, in which the label is a luminescent molecule and binds to the specified hormone, can accurately identify serum hormone levels including AMH, LH, FSH, estrogen, progesterone, prolactin and sex hormone binding globulin (SHBG) from a blood sample after incubation and washout period [193]. The luminometer

spectrophotometrically detects the cleavage of the chemiluminescent substrate [193]. The amount of photon produced can be utilized to determine the concentration of the hormone [193].

3.2.2 Exposure and Comparator:

The exposure for this study is COS treatment. Participants were studied at the peak of COS treatment, following 7-10 days of gonadotropin injection. The comparator was the participant's baseline, assessed immediately prior to the initiation of COS treatment. The exposure and comparator were assessed on two separate study visits, the comparator visit occurred immediately prior to initiation of COS treatment (on day 1-3 of the menstrual cycle, immediately prior to initiation of gonadotropins) and the exposure visit occurred at the peak of COS treatment (on day 7-10 of the COS treatment) (see Figure 6). At each study visit, each outcome was measured and a comparison of the outcomes between study visits was completed. To account for confounding due to variations in dosing of exogenous hormone(s) and levels of endogenous hormones (i.e., in response to COS) during exposure, each participant acted as their own comparator.

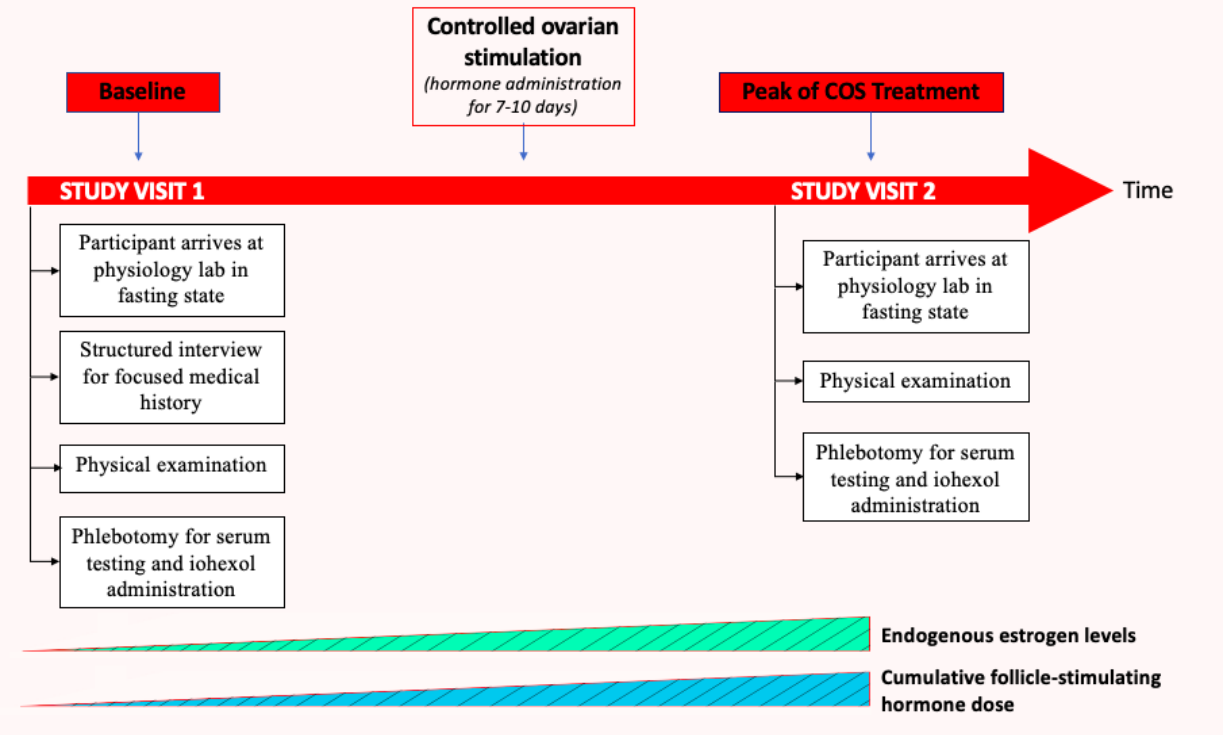

Figure 6. The study design demonstrating study visits for exposure and comparator data collection. Adapted and reprinted with permission from Elaha Niazi.

3.2.3 Measurement of Outcomes

3.2.3.1 measured Glomerular Filtration Rate (mGFR)

GFR is the rate in milliliters per minute at which substances in plasma are filtered through the glomerulus [194], and it was measured using the administration of iohexol. An 18-gauge peripheral venous cannula was inserted into the antecubital vein (for iohexol administration and for phlebotomy) and each participant received a bolus dose of 1500 mg of iohexol over 2 minutes. Blood draws were completed seven times following the iohexol bolus, starting after 60 minutes and then in 30 minutes increments until 240 minutes (Figure 7). To determine the plasma clearance of iohexol, the concentration of plasma iohexol from each blood draw was measured using Liquid Chromatography Tandem Mass Spectroscopy (Sciex 6500+ Qtrap) with a coefficient of variation

of <3%, at the University of Minnesota. Iohexol concentrations for each time point were plotted on a logarithmic graph (Figure 8). From the plotted points, 2 curves can be identified (fast component curve and slow component curve) and the second curve (slow component curve), which corresponds to the kidney clearance of iohexol, can be used to calculate mGFR [82]. Specifically, mGFR is calculated using both the iohexol dose and the area under the curve (AUC) of the slow component curve [82].

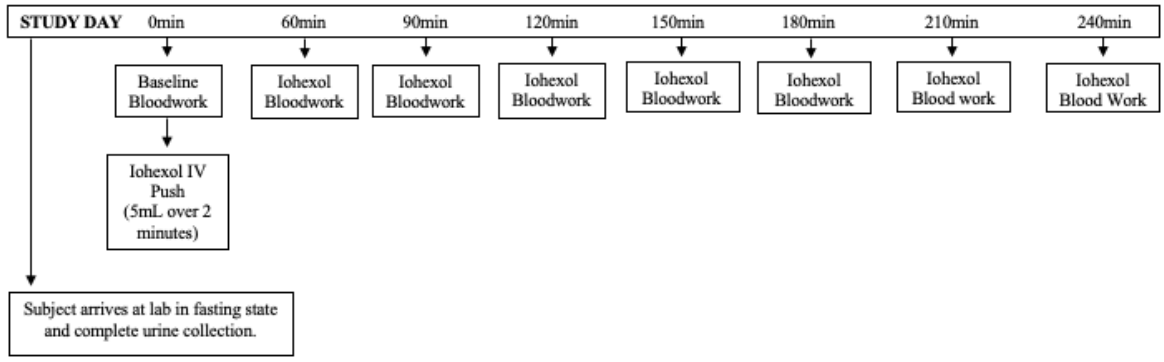

Figure 7. General Study Protocol outlining measurement of study outcomes.

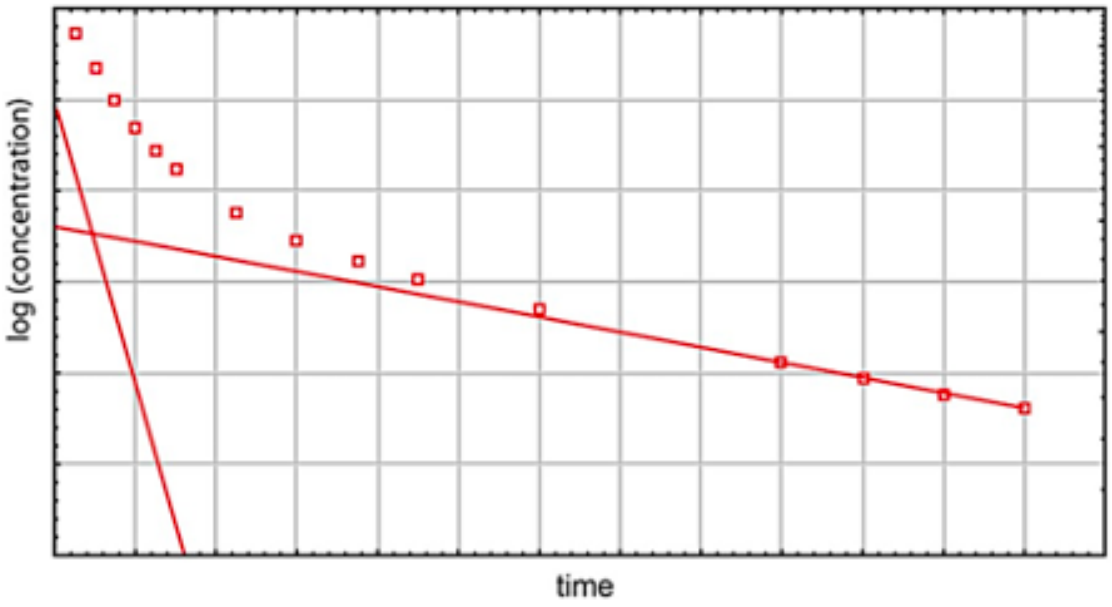

Figure 8. Example plot of iohexol elimination from plasma after a single bolus infusion of iohexol [82].

3.4.2 Albuminuria-Creatinine ratio (ACR)

The albumin to creatinine ratio (ACR) is defined as the albumin concentration divided by the creatinine concentration in a single urine sample [92] . To collect urine for this test, a spot urine sample was collected midstream [195-197] and transferred to the laboratory for testing. To quantify the urinary albumin and creatinine concentrations, immunoturbidimetric assays on Cobas c702 and c502 were used by Alberta Precision Laboratories. In this method, anti-albumin antibodies bind to the antigens in the sample material and create an antigen-antibody complex which are further measured turbidimetrically at 340nm [92]. The urinary albumin and creatinine concentrations are then used to calculate the ACR [92, 195].

3.5 General Protocol

Individuals that expressed interest in the study from the variety of recruitment methods above were asked to contact the research study coordinator through phone or email. Following initial contact, the study coordinator arranged a brief phone call to assess eligibility and outline study procedures. Eligible participants were invited to join the study by participating in 2 visits to the physiology laboratory. Informed consent was obtained at Study Visit 1 using a consent document and a personalized conversation with a research nurse regarding any questions or concerns about study participation. Participants visited the research laboratory on two study days: the first study day occurred at baseline prior to initiating COS treatment in the early follicular phase of their menstrual cycle, and the second study day occurred at the peak of controlled ovarian stimulation (usually between day 7-10 of COS treatment) (Figure 6). For both study visits, participants were instructed to undergo a 12-hour fast and abstain from alcohol, smoking, caffeine, and recreational drugs for at least four hours prior to each study visit, given that these factors may influence the measurement

results [198-203]. Regularly scheduled medications could continue for this time period with sips of water. Through a semi-structured interview, each participant provided baseline demographic and medical information on the first visit. Physical examination, laboratory, and outcome measures were collected at both visits. First, participants provided a urine sample. Then, a physical examination was conducted, and demographic and medical history was collected. Finally, participants were instructed to lay in a supine position in a quiet, temperature-controlled room to prepare for phlebotomy and measurement of study outcomes. An 18-gauge peripheral venous cannula was inserted into the antecubital vein (for iohexol administration and for phlebotomy) and each participant received a bolus of 1500 mg of iohexol Regular blood draws occurred every 30 minutes starting 60 minutes following iohexol bolus (Figure 7). To ensure consistency in data collection, a Registered Nurse with experience in conducting physiological studies was responsible for collecting the study data and outcome measurements.

3.5.1 Sex and Gender Considerations

COS treatment is only available to individuals who are biologically female, so this was a single-sex study. However, we designed this study specifically to recruit individuals from diverse gender identities. The language, design and symbols for our recruitment materials, data collection form, and resources for knowledge mobilization were created to encourage participation of individuals from a wide spectrum of gender identity. Further, the manuscript written based on this traditional thesis will be prepared in accordance with the SAGER guidelines [204].

3.6 Statistical Analysis

Baseline descriptive and categorical data, stratified by study visit, are presented as a median and interquartile ranges (IQR) or percentages. The primary outcome in this study is a comparison of each outcome (GFR and ACR) between the baseline (Study Visit 1) and during COS (Study Visit 2) and was evaluated with the non-parametric, Wilcoxon signed rank test. Physical examination parameters and laboratory values between study visits were also compared using Wilcoxon signed rank tests. Sensitivity analyses were conducted to assess the robustness of the results, by 1) excluding participants treated with COS for oocyte cryopreservation, 2) excluding participants who are not treated with the antagonist protocol for COS treatment, 3) excluding participants that had been treated with COS previously, 4) excluding participants with chronic kidney disease or other chronic disease, 5) excluding participants >35 years. A significance level for all tests was set at $p<0.05$. All statistical analyses were performed using STATA version 18.0 (StataCorp, Texas).

3.6.1 Sample Size Calculation

We aimed to recruit a convenience sample that targeted at least 20 females undergoing COS treatment. Given the exploratory nature of this prospective observational study, a sample size calculation was not feasible.

CHAPTER FOUR: RESULTS

4.1 Participant Characteristics

Participant characteristics at baseline are listed in Table 1. In total, 10 individuals met the study criteria and were enrolled in the study. The median age of participants was 35 years, ranging from 27 to 39 years. The self-described ethnicity distribution of the participants constituted primarily of White individuals (80%), with a minority identifying as including Latinx (10%), Southeast Asian (10%), and Caribbean (10%). Most individuals described their sexual orientation as straight (80%), with others identifying as lesbian (10%) or queer (10%). All participants identified as women and the median neighborhood income was determined based on their postal code; 60% of the participants lived in neighborhoods with median income greater than $100,000 and 40% of participants lived in neighborhoods with median income less than $100,000. The median weight and abdominal circumference were 63 kg (IQR: 9) and 73 cm (IQR: 6), respectively. When assessing the baseline BMI, a large population of patients fell in the normal BMI range of 18.5 – 24.9 kg/m^2; however, 10% were underweight (< 18.5 kg/m^2) and 10% were obese class III (i.e, ≥ 40 kg/m^2). Current cigarette smoking was uncommon (10%) and half of participants reported medical comorbidities. Half of participants reported symptoms associated with depression and/or anxiety. Regular daily NSAID use was uncommon (10%).

Table 1. Baseline Participant Characteristics	
	n = 10
Age (years)	35 (3)
Ethnicity[a]	
Caribbean	1 (10%)
Latinx	1 (10%)
Southeast Asian	1 (10%)
White	8 (80%)
Sexual orientation	
Heterosexual/Straight	8 (80%)
Lesbian	1 (10%)
Queer	1 (10%)
Gender	
Woman	10 (100%)
Median neighbourhood income	
< $100,000	6 (60%)
≥ $100,000	4 (40%)
Weight (kg)	63 (9)
BMI (kg/m^2)	
<18.5	1 (10%)
18.5 – 24.9	8 (80%)
25.0 – 29.9	0 (0%)
30.0 – 34.9	0 (0%)
35.0 – 39.9	0 (0%)
≥ 40	1 (10%)
Abdominal circumference (cm)	73 (6)
Current Cigarette Smoking	
Yes	1 (10%)
Medical Co-morbidities	
Cancer	1 (10%)
Cardiovascular Disease Cardiac Arrythmia	1 (10%) 1 (10%)
Polycystic Ovarian Syndrome	3 (30%)
Self-reported depression and/or anxiety	
Yes	4 (40%)
Maybe	1 (10%)
Daily NSAID Use	1 (10%)

Data are presented as median (IQR) or number of participants (percentage).

[a]Proportions/percentages do not add up to 100% as participants were able to self-identify as

multiple ethnicities. Abbreviations: BMI = Body Mass Index, NSAIDS = Non-steroidal anti-inflammatory drugs.

4.1.1 Reproductive Characteristics

Reproductive characteristics of participants are outlined in Table 2. The majority of the participants were nulligravid (60%) and nulliparous (70%). Only 1 participant reported having a spontaneous abortion, and 2 participants reported previous pregnancy complications, including right fallopian tube rupture and mental health adjustment disorder. The causes of infertility varied among participants and included male-factor infertility (50%), male- and female-factor infertility (10%), unexplained infertility (30%) and elective oocyte preservation (10%). Some participants underwent previous fertility treatment including intrauterine insemination (IUI) (30%) or ovulation induction (30%). Few participants had been previously treated with COS (1 previous cycle) (30%). Table 3 demonstrates that participants were on a variation of protocols such as antagonist (70%), antagonist with combined oral contraceptive pill (20%) and estrogen priming (10%).

Table 2. Baseline Participant Reproductive Characteristics	
	n=10
Gravidity	
0	6 (60%)
1	4 (40%)
Parity	
0	7 (70%)
1	3 (30%)
Previous Pregnancy Loss	
Spontaneous abortion	1 (10%)
Pregnancy complications	
Right fallopian tube rupture	1 (10%)
Mental health adjustment disorder	1 (10%)
Cause of infertility	
Elective oocyte preservation	1(10%)
Female factor	0 (0%)
Male factor	5 (50%)
Female + Male Factor	1 (10%)
Unexplained	3 (30%)
Previous fertility treatment	
Intrauterine insemination	3 (30%)
Ovulation induction	3 (30%)
Previous COS treatment	
0 cycle	7 (70%)
1 cycle	3 (30%)

Data are presented as number of participants (percentage). Abbreviations: COS = Controlled ovarian stimulation.

Table 3. Type of COS Protocol for Study Participants	
	n=10
Antagonist	7 (70%)
Antagonist/OCP	2 (20%)
Estrogen priming	1 (10%)

Data are presented as number of participants (percentage). Abbreviations: COS = Controlled ovarian stimulation, OCP = Oral contraceptive pill.

4.2 Change in Serum Hormone Levels Between Pre- and Post-COS Study Visits

Table 4 describes the change in serum hormone levels between participants' baseline study visit and exposure study visit (at the peak of COS treatment). The median AMH levels at baseline were 27.6 (22.3) pmol/L and at post-COS 10.4 (3.3) pmol/L. The difference between pre- and post-COS AMH levels was not significant (p=0.07). The median estrogen levels at baseline were 225(182) pmol/L and at the peak of COS treatment were 7232.5(4259) pmol/L. The increase in estrogen levels observed between pre- and post-COS was statistically significant (p=0.03). The median percent change in estrogen level between study day 1 and study day 2 was 3830%, and the smallest percent change was 1925% while the largest observed was 8915%. The median FSH levels at baseline were 5.9 (2.7) IU/L and at peak of COS treatment were 14 (10.9) IU/L. The increase in FSH levels were statistically significant (p=0.03). The median LH levels were calculated as 5.6 (2) IU/L and at peak of COS treatment were 3.05 (13.3) IU/L. These changes observed were not found to be statistically significant (p=0.89). Next, the median progesterone levels observed were 0.95 (0.4) nmol/L at baseline and 3.35 (8.6) nmol/L at peak of COS treatment. The changes observed did not result in a statistically significant difference (p=0.35). The median levels of prolactin increased significantly from 9.35 (5.6) ug/L at baseline to 30.6 (23.45) ug/L at peak of COS treatment (p=0.03). Finally, the median levels of SHBG pre-COS were 95.5 (81) nmol/L and post-COS were 100(44) nmol/L with no significant differences observed (p=0.44).

Table 4. Change in Serum Hormone Levels Between Pre- and Post-COS Study Visits			
Hormones	**Pre-COS**	**Post-COS**	**p-value**
AMH (pmol/L)	27.6 (22.3)	10.4 (3.3)	0.07
Estrogen (pmol/L)	225 (182)	7232.5 (4259)	**0.03**
FSH (IU/L)	5.9 (2.7)	14 (10.9)	**0.03**
LH (IU/L)	5.6 (2)	3.05 (13.3)	0.89
Progesterone (nmol/L)	0.95 (0.4)	3.35 (8.6)	0.35
Prolactin (ug/L)	9.35 (5.6)	30.6 (23.45)	**0.03**
SHBG (nmol/L)	95.5 (81)	100 (44)	0.44

Data are presented as median (IQR). This data is from a partial sample, 2 participants were excluded due to insufficient data. Abbreviations: AMH = Anti-Müllerian hormone, COS = Controlled ovarian stimulation, FSH = Follicle stimulating hormone, LH = Luteinizing hormone, SHBG = Sex-hormone binding globulin.

4.3 Change in Participant Cardiometabolic Characteristics Between Pre- and Post-COS Study Visits

In Table 5, changes in cardiometabolic characteristics including weight (kg), abdominal circumference (cm), average systolic blood pressure (avg. SBP) and average diastolic blood pressure (avg. DBP) were presented as median percent changes between pre-COS and post-COS study visits. Interestingly, there was a statistically significant increase in weight between pre- and post- COS study visits *(p*=0.005), that ranged between 0.31 – 4% with a median increase of 0.85%. No significant changes in abdominal circumference or blood pressure were evident.

Table 5. Change in Participant Cardiometabolic Characteristics Between Pre- and Post-COS Study Visits		
	Median percent change (Range)	**p-value**
Weight (kg)	0.85 (0.31 – 4.00)	**0.005**
Abdominal circumference (cm)	1.95 (-4.17 – 6.85)	0.22
Avg. SBP	0.16 (-20.72 – 8.5)	0.76
Avg. DBP	-2.44 (-23.55 – 9.42)	0.54

Abbreviations: COS = Controlled ovarian stimulation, Avg SBP = Average systolic blood pressure, Avg DBP = Average diastolic blood pressure.

4.4 Primary Outcomes

4.4.1 Measured Glomerular Filtration Rate (mGFR)

Each participant's mGFR at pre-COS and post-COS study visits, as well as each participant's percent change in mGFR, is outlined in Table 6. Most participants demonstrated an increase in mGFR between the pre-COS and post-COS study visit, with a median change of 8% and a range of -5% to 37% (Figure 9). Despite a trend towards an increased mGFR, no statistically significant differences ($p = 0.13$) were observed in participants' mGFR at baseline compared to mGFR at the peak of COS treatment (Figures 10 and 11). However, upon examining individual participant data, 71% of the participants demonstrated an increase in their mGFR between pre-COS and post-COS, while 29% of the participants had a decrease in mGFR between pre-COS and post-COS (Table 6, Figure 11).

Table 6. Percent Change in measured Glomerular Filtration Rate (mGFR) between Pre- and Post-COS Study Visits

	Pre-COS mGFR (mL/min/1.73 m^2)	**Post-COS mGFR**(mL/min/1.73 m^2)	**Absolute Change in mGFR (**mL/min/1.73 m^2)	**Percent Change in mGFR (%)**
Participant A	83	90	7	8
Participant B	83	103	20	23
Participant C	95	110	15	16
Participant D	70	95	25	37
Participant E	76	72	-4	-5
Participant F	104	104	0	0.4
Participant G	87	83	-4	-4
Median % change	**8 (Q1:-4, Q3: 23) Range: (-5, 37)**			

Data are presented as median (IQR) for pre- and post-COS mGFR. This data is from a partial sample, 3 participants were excluded due to insufficient data. Abbreviations: COS = Controlled ovarian stimulation, mGFR = Measured glomerular filtration rate.

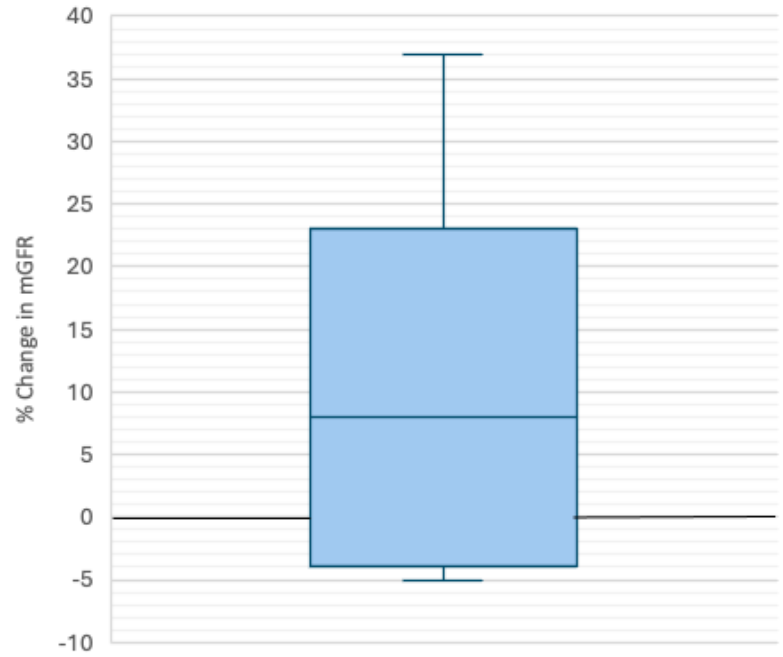

Figure 9. Box and whisker plot demonstrating the percent change in measured GFR. Abbreviations: mGFR: Measured glomerular filtration rate.

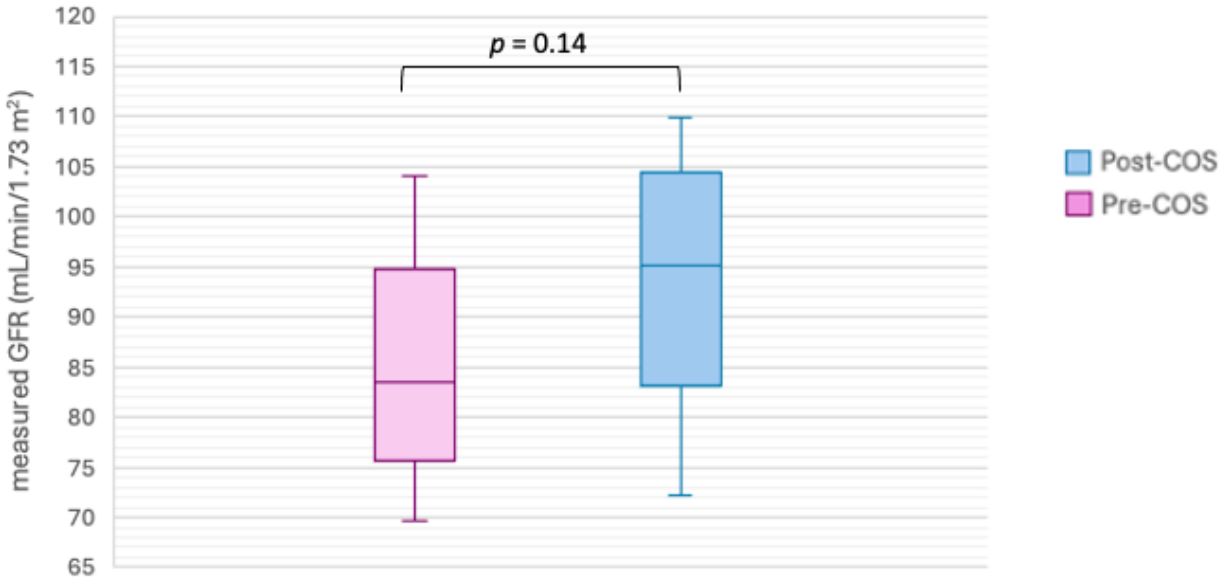

Figure 10. The mGFR at pre-COS (baseline) and post-COS treatment study visits, by study sample. Abbreviations: mGFR = Measured glomerular filtration rate, COS = Controlled ovarian stimulation.

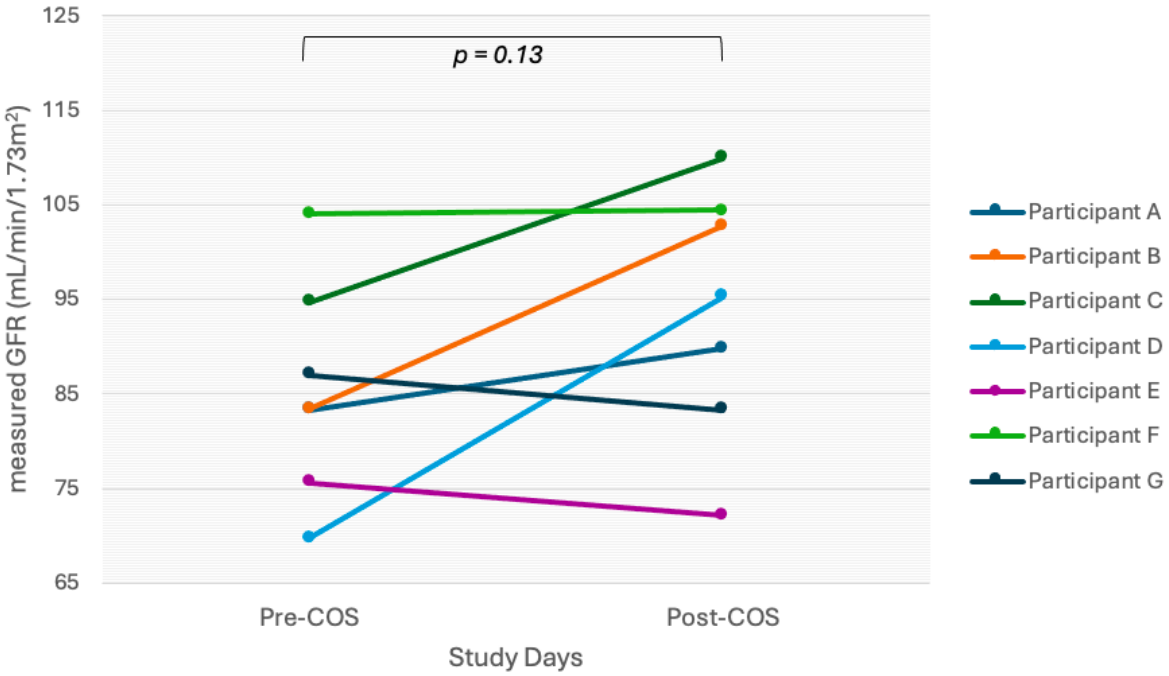

Figure 11. The mGFR at the pre-COS (baseline) and post-COS treatment study visits, by study participant. Abbreviations: mGFR = Measured glomerular filtration rate, COS = Controlled ovarian stimulation.

4.4.2 Albumin-Creatinine-Ratio (ACR)

Each participant's ACR at pre-COS and post-COS study visits, as well as each participant's percent change in ACR, is outlined in Table 7. Most participants demonstrated a decrease in mGFR between the pre-COS and post-COS study visit, with a median change of -44% and a range of -100% to 207% (Figure 12). Overall, the difference in ACR between pre- and pos-COS study visits was not statistically significant (p = 0.89) (Figures 13 & 14). On examination of individual participant data, 67% of participants demonstrated a decrease in ACR between pre and post COS and 33% demonstrated an increase in ACR pre and post COS (Table 7, Figure 13).

Table 7. Percent Changes in Albumin-Creatinine-Ratio (ACR) between Pre- and Post-COS Study Visits				
	Pre-COS ACR mg/mmol)	**Post-COS ACR (**mg/mmol)	**Absolute Change in ACR (mg/mmol)**	**Percent Change in ACR (%)**
Participant A	0.63	0.91	0.28	44
Participant B	1.18	0.62	-0.56	-48
Participant C	0.31	0.00	-0.31	-100
Participant D	0.23	0.61	0.38	165
Participant F	0.40	0.38	-0.02	-5
Participant G	0.95	2.92	1.97	207
Participant H	0.88	0.33	-0.55	-63
Participant I	1.76	0.59	-1.17	-67
Participant J	0.88	0.49	-0.39	-44
Median % Change	**-44 (Q1:-63, Q3: 44) Range: (-100, 207)**			

Data are presented as median (IQR) for pre- and post-COS ACR. This data is from a partial sample, 1 participant was excluded due to insufficient data. Abbreviations: COS = Controlled ovarian stimulation, ACR = Albumin-creatinine-ratio.

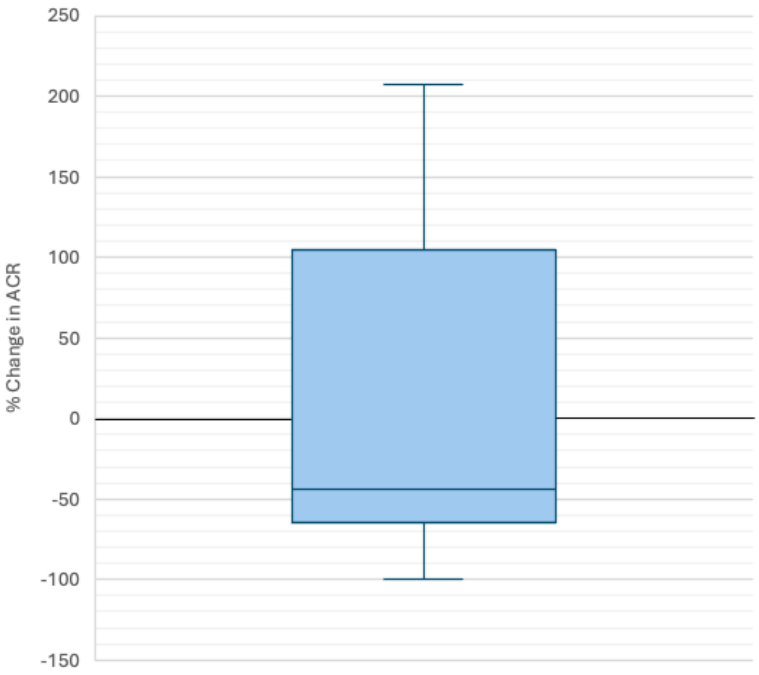

Figure 12. Box and whisker plot demonstrating the percent change in measured ACR (mg/mmol). Abbreviations: ACR = Albumin-creatinine-ratio.

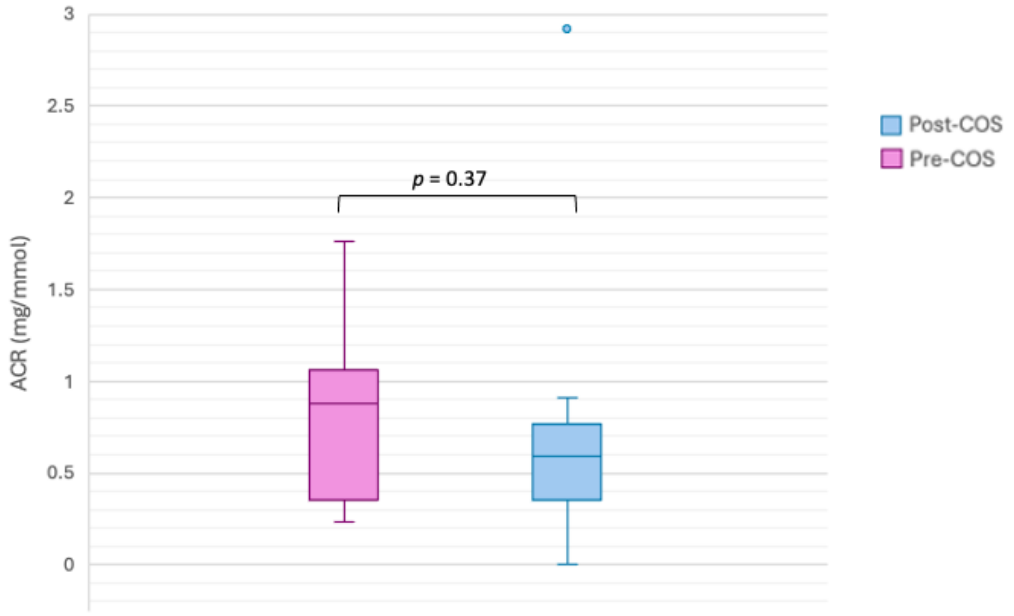

Figure 13. The ACR at the baseline (pre-COS) and post-COS treatment study visits, by study sample. Abbreviations: ACR = Albumin-creatinine-ratio, COS = Controlled ovarian stimulation.

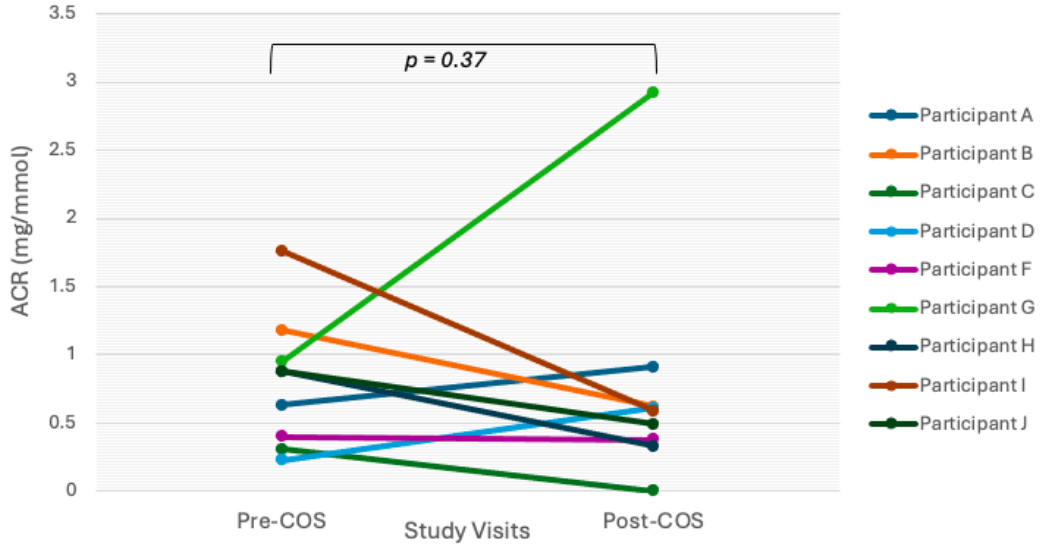

Figure 14. The ACR at the baseline (pre-COS) and post-COS treatment study visits, by participant. Abbreviations: ACR = Albumin-creatinine-ratio, COS = Controlled ovarian stimulation

4.5 Additional Exploratory Analyses

Table 8 provides a summary of the COS characteristics and kidney function outcomes by each individual participant. Most participants were on an antagonist protocol (70%). The number of days that FSH was administered prior to study visit 2 ranged from 7- 10 in all participants, and most participants had 8 or 9 days of FSH administration prior to study visit 2 (80%). The cumulative dose of FSH during COS ranged between 1100 – 3600 IU, with a median dose of 1675 IU (Figure 15). Additionally, estrogen levels measured on study day 2 ranged between 2,487 – 16,227 pmol/L, with a median level of 7232.5 pmol/L (Figure 16). The median number of oocytes retrieved from each participant was 18, 70% participants had more than 15 oocytes available for retrieval with 30% of participants with ≤10 oocytes available for retrieval. Upon examination of individual participant data, no obvious differences or patterns in these measured COS characteristics exist between individuals with increased vs decreased mGFR or increased vs decreased ACR.

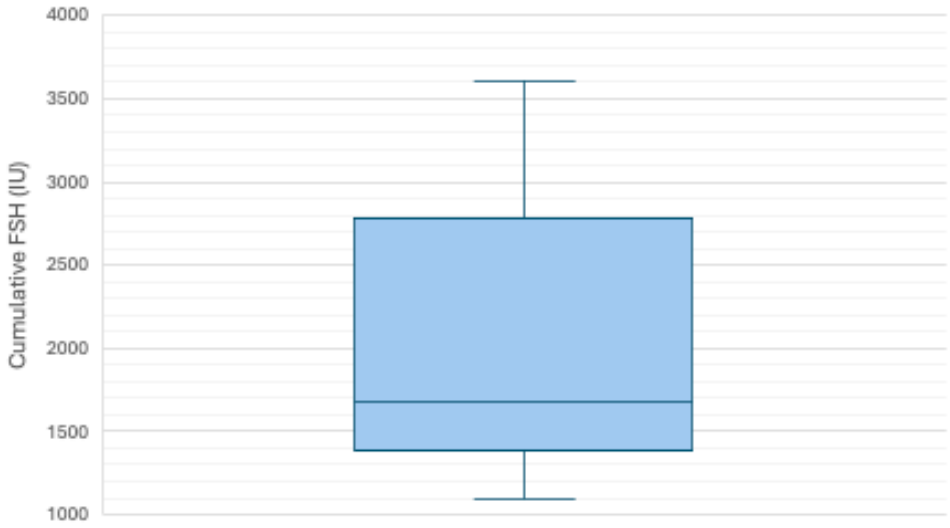

Figure 15. Box and whisker plot demonstrating the cumulative dose of FSH (IU) administered during COS treatment. Abbreviations: FSH = Follicle-stimulating hormone.

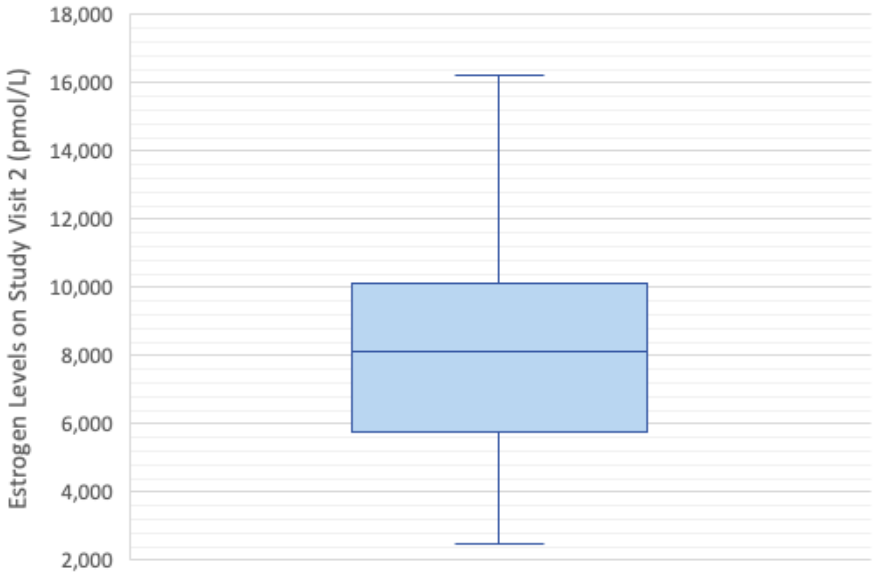

Figure 16. Box and whisker plot demonstrating the elevation in estrogen level (pmol/L) after treatment with COS (i.e., study visit 2). Abbreviations: COS = Controlled ovarian stimulation.

Table 8. COS Characteristics and Kidney Function Outcomes for Each Participant									
Participant	**Type of COS Protocol**	**Number of Days of FSH Administration at Study Visit 2**	**Cumulative FSH Dose (IU)**	**Estrogen Level on Study Visit 2 (pmol/L)**	**Oocytes Retrieved**	Absolute Change in mGFR	**% Change in mGFR**	Absolute Change in ACR	**% Change in ACR**
Participant A	Antagonist	9	1325	10,498	18	7	8	0.28	44
Participant B	Antagonist	9	1550	9,276	26	20	23	-0.56	-48
Participant C	Antagonist /OCP	10	2650	4,540	16	15	16	-0.31	-100
Participant D	Antagonist	8	1800	6,808	21	25	37	0.38	165
Participant E	Antagonist	7	3150	7,657	4	-4	-5	N/A	N/A
Participant F	Antagonist /OCP	8	1100	2,487	18	0	0.4	-0.02	-5
Participant G	Antagonist	8	1400	9,984	21	-4	-4	1.97	207
Participant H	Estrogen priming	8	2400	6,202	8	N/A	N/A	-0.55	-63
Participant I	Antagonist	9	1475	16,227	21	N/A	N/A	-1.17	-67
Participant J	Antagonist	8	3600	8,564	10	N/A	N/A	-0.39	-44
Median (IQR)	-	**8 (1)**	**1675 (1250)**	**8111 (3782)**	**18 (11)**	**7 (24)**	**8 (28)**	**-0.31 (0.83)**	**-44 (107)**

Insufficient data is indicated with N/A. Abbreviations: COS = Controlled ovarian stimulation, OCP = Oral contraceptive pill, FSH = follicle-stimulating hormone, mGFR = Measured glomerular filtration rate, ACR = Albumin-creatinine-ratio.

A linear regression analysis was completed to formally assess the relationship between the participant's percent change in estrogen level and the percent change in mGFR (Figure 17). No significant relationship was identified (R2=0.01, *p*=0.85). Another linear regression analysis formally assessed the relationship between the participant's cumulative FSH dose and the percent change in mGFR (Figure 18). No significant relationship was identified (R2=0.01, *p*=0.85).

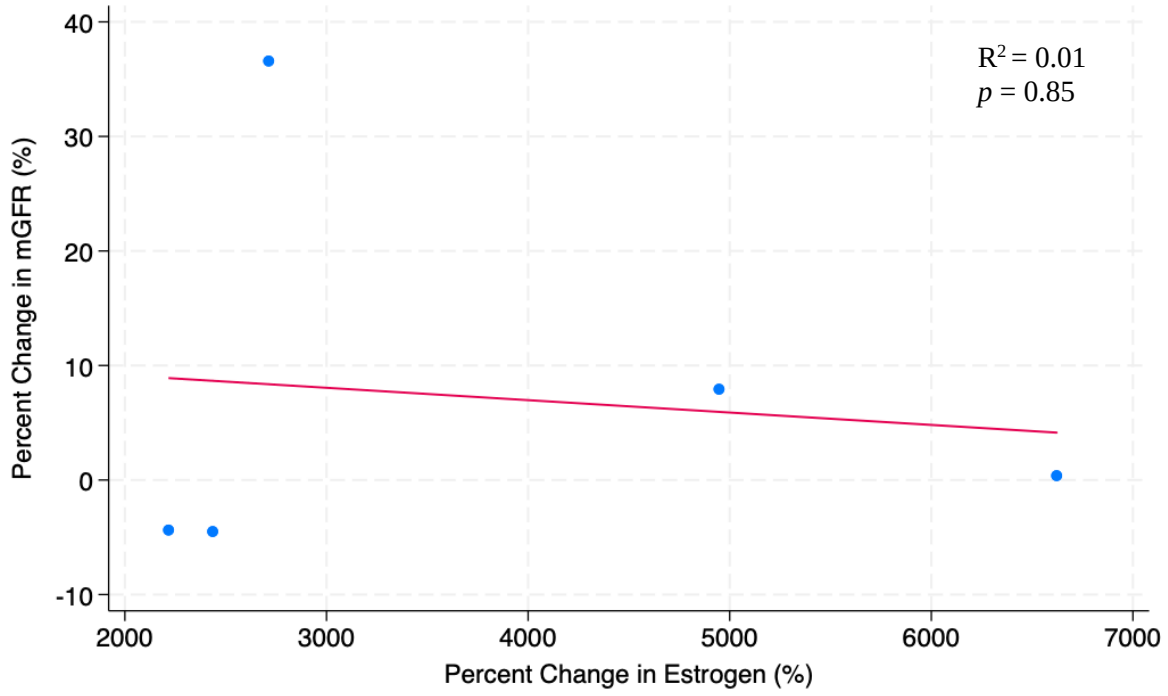

Figure 17. The association between participant's percent change in estrogen level and percent change in measured GFR. Abbreviations: mGFR = Measured glomerular filtration rate.

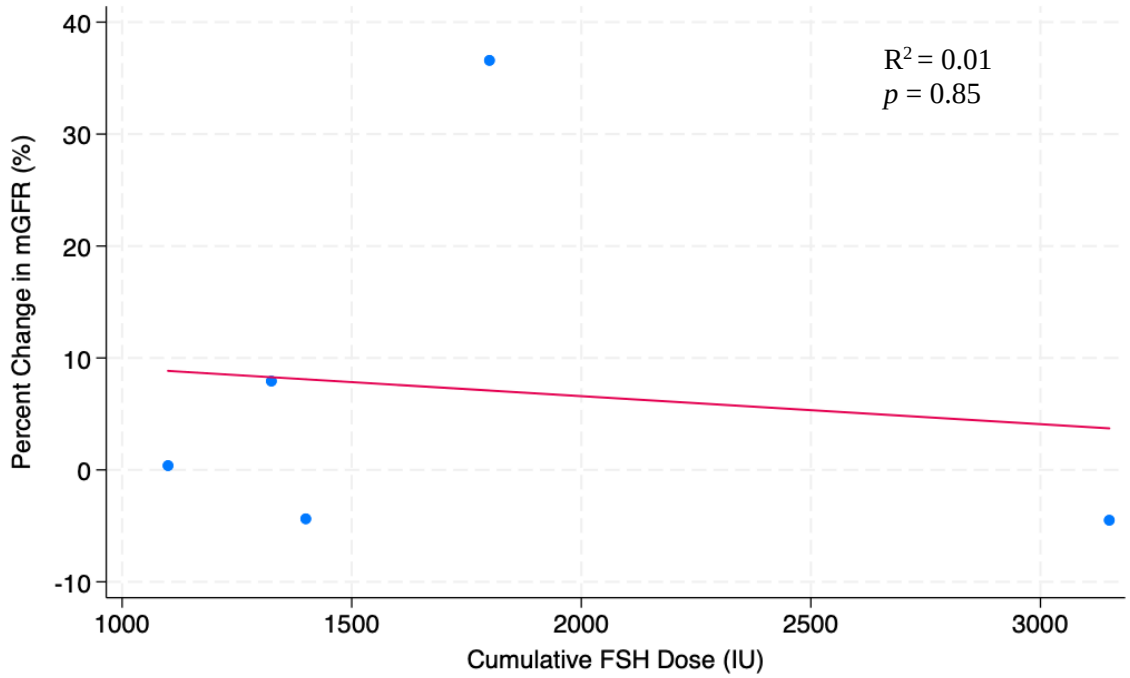

Figure 18. The association between participant's cumulative FSH dose and percent change in measured GFR. Abbreviations: mGFR = Measured glomerular filtration rate, FSH = Follicle-stimulating hormone.

A linear regression analysis was completed to formally assess the relationship between the participant's percent change in estrogen level and the percent change in ACR (Figure 19). No significant relationship was identified, though a trend towards a negative relationship was noted (R2=0.35, *p*=0.16). Another linear regression analysis formally assessed the relationship between the participant's cumulative FSH dose and the percent change in ACR (Figure 20). No significant relationship was identified (R2=0.13, *p*=0.42).

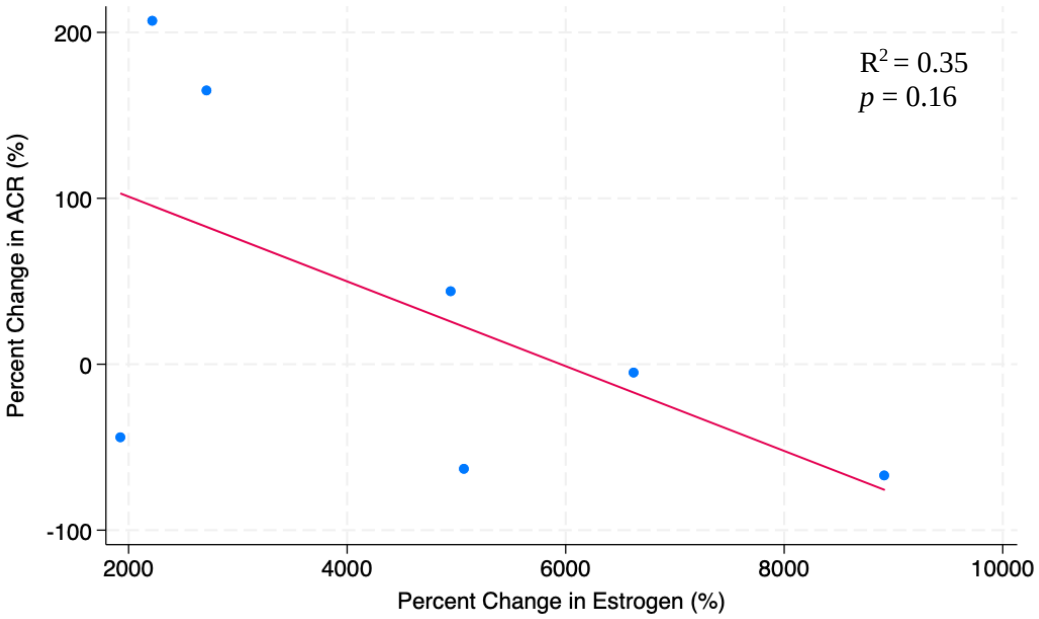

Figure 19. The association between participant's percent change in estrogen level and percent change in ACR. Abbreviations: ACR = Albumin-creatinine-ratio.

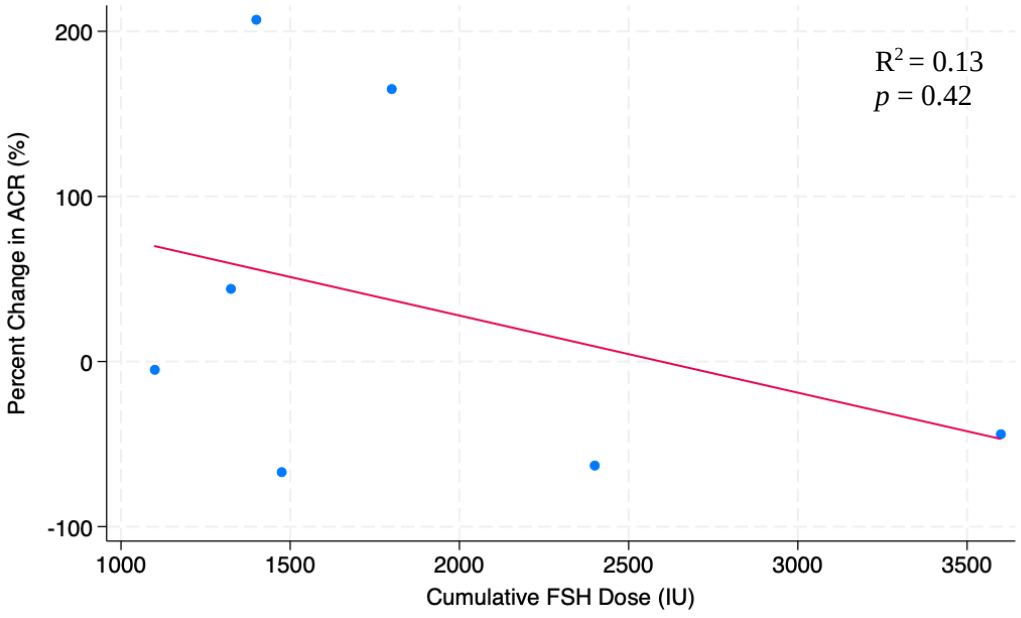

Figure 20. The association between participant's cumulative FSH dose (IU) and percent change in ACR. Abbreviations: ACR = Albumin-creatinine-ratio, FSH = Follicle-stimulating hormone.

4.6 Sensitivity Analyses

Five separate sensitivity analyses, in which we 1) excluded participants who were doing oocyte cryopreservation, 2) excluded participants who were on a non-antagonist protocol (i.e., estrogen priming, oral contraceptive pill etc.), 3) excluded participants that have been treated with COS previously, 4) excluded participants with chronic kidney disease or other chronic disease (i.e., cancer, PCOS etc), 5) excluded participants >35 years did not result in any differences in study results.

CHAPTER FIVE: DISCUSSION

The purpose of this prospective observational study was to determine the effect of COS on kidney function, including mGFR and ACR, in females treated with COS. Overall, this study demonstrated that no significant change in mGFR and ACR was observed related to COS, though a trend towards an increase in mGFR and reduction in ACR was noted. Further, a trend towards a negative relationship between percent change in estrogen level and percent change in ACR was observed. Recognizing the small sample size resulting in limited study power, these findings suggest that COS may impact kidney function.

Currently, there is a gap in literature addressing the impact of COS on healthy kidney function. However, it is well understood that exogenous and endogenous sex hormones effect kidney function [31, 99-102, 106-108, 116, 118, 121, 130]. The majority of the literature to date focuses on endogenous and exogenous estrogen, in the form of menopause and hormone treatment (PHT, OCP, GAHT), respectively [98, 105, 116, 118, 120]. Endogenous estrogen has been reported to have nephroprotective properties in females [100, 101, 105]. Previous research that has examined the effect of estrogen-containing hormone administration (through oral contraceptives and post-menopausal hormone therapy) has produced conflicting results. Some studies have found an increase in GFR [205], while others have found a decrease or no difference in GFR [114, 115, 118, 120, 206]. In our study, we found no significant difference between pre-COS and post-COS mGFR, indicating no clear effect of COS on mGFR, however, we observed a trend towards an increase in mGFR. Interestingly, this trend towards an increased mGFR did not appear to be related to either the increase in estrogen level or cumulative FSH dose. It is important to note that our study population were healthy individuals with no CKD and mGFR measurements that fell into

the healthy range. Similarly, when assessing ACR, previous studies examining the relationship between estrogen-containing hormone treatment and kidney function also found inconsistent results. Most studies found an increase in albumin excretion associated with hormone use [115, 116, 118, 207], but some found a decrease or no difference in ACR [114, 205, 206, 208-210]. According to the results of our study, there was no significant difference between pre-COS and post-COS ACR measurements, though a trend toward a decrease in ACR was identified. Of special interest, the decrease in ACR was closely related to the participants' increase in estrogen level (but not cumulative FSH dose). It is important to mention that despite large percent changes in ACR in the study population, all ACR readings remained in the healthy range.

There is no literature that can serve as a direct comparison to assess for the reliability of our results, as this is the first study that examines the impact of COS on healthy kidney function. A notable trend that indicates that COS may increase mGFR and reduce ACR was observed, though it didn't reach statistical significance. We recognize that this study is likely underpowered, related to the small sample size, and that these potential effects may be more apparent in a larger study. Interestingly, increases in GFR (and specifically hyperfiltration) is commonly observed in conjunction with an increase in albuminuria [211-213]. In our study, interestingly, a non-significant trend towards concurrently increased mGFR and reduced ACR were demonstrated.

Glomerular hyperfiltration is difficult to define, but according to several studies, the threshold for glomerular hyperfiltration ranges from 125 ml/min/1.73 m^2 to 175 ml/min/1.73 m^2 [214]. In our study, we found that 70% of participants had an increase in their mGFR between study visits, but no mGFR readings were within this range of hyperfiltration. During pregnancy, an individual's

GFR increases by 40-65% within 1 month of conception and persists through pregnancy [215, 216]. It is unclear what mechanism is responsible for this effect in pregnancy [217], though it has been postulated to be related to elevations in estrogen [218]. In our study, the increase in mGFR was not associated with the increase in estrogen level, which suggests that other potential factors may be at play.

Albuminuria is a significant predictor of kidney and cardiovascular outcomes [219-221], and evidence suggests that reductions in albuminuria are associated with fewer kidney and cardiovascular events [222]. In our study, we observed a non-significant trend towards a decrease in albuminuria, which suggests a potential relationship between COS and ACR. Of interest, the reduction in ACR appeared to correlated with an increased level of estrogen, suggesting a relationship between the 2 measures, similar to what is observed in individuals using hormone treatments (PHT, OCP) effect [114-118, 120]. Studies with larger sample sizes are required to further solidify our understanding of the relationship between ACR and changes in estrogen levels. Although in our study, all ACR measurements were within the healthy range (<3 mg/mmol)[2], it is important to recognize that incremental reductions within this range may also be beneficial [223].

Our study observed that changes in hormone levels occurred between baseline and post-COS study visits. Specifically, levels of estrogen (p=0.03), FSH (p=0.03) and prolactin (p=0.03) increased significantly. The median estrogen levels before initiation of COS were 225 (182) pmol/L and post- COS were 7232.5 (4259) pmol/L, more than 30 times higher than pre-COS. This finding was expected and aligns with previous literature that found that serum estradiol levels may increase

more than 20 times [177]. Serum estradiol increases as a result of the administration of FSH, which results in increased growth of ovarian follicles and therefore, increased follicular secretion of estradiol [224]. Similarly, the median FSH levels at baseline were 5.9 (2.7) IU/L and post-COS were 14 (10.9) IU/L, which likely relates to the high doses of exogenous FSH administered during COS [225]. In terms of prolactin, we found a significant increase in the median prolactin levels during COS. Previous studies have also reported hyperprolactinemia during COS [226-228], and prolactin increase during COS related treatments has been suggested as a consequence of stress since it is considered a stress hormone [45]. Interestingly, increasing stress hormones during COS may be tied to symptoms of anxiety [229].

In this study, we assessed differences in cardiometabolic parameters including weight, abdominal circumference, average systolic blood pressure, and average diastolic blood pressure between pre- and post-COS. We observed a median percent increase of 0.85% body weight, ranging between 0.31% to 4%, between the pre- and post-COS study visits (p=0.005). Previous studies have likewise observed a significant change in weight for individuals treated with COS [230-233]. It remains unclear what this weight gain may represent, but fluid, fat mass, and constipation are all proposed mechanisms.

This study also examined the impact of the cumulative FSH dose on the percent change in mGFR between pre- and post-COS study visits. There was no significant correlation between FSH dose and changes in mGFR (p=0.85). This was an interesting finding within the context of previous literature that demonstrates a negative correlation between FSH and eGFR. It is important to remember, however, that FSH level does not necessarily reflect dosing of FSH in COS.

Lastly, we conducted sensitivity analyses and found no significant differences in our results. First, we excluded participants who were treated with COS for oocyte cryopreservation (not for IVF) to identify whether any non-COS factors (such as participant diagnosis of infertility) may result in different outcomes. Secondly, we excluded participants who were on a non-antagonist protocol (i.e., estrogen priming, oral contraceptive pill etc.), as these protocols premedicated the participants with estrogen, which may have impacted our initial estrogen levels and therefore our change in estrogen level between study visit 1 and 2. Additionally, we excluded participants that have been treated with COS previously. Limited studies that have demonstrated cumulative effects of multiple COS cycles on cardiovascular outcomes, and it is unclear whether this could apply to kidney function outcomes. We also aimed to exclude participants with chronic kidney disease or other chronic disease (i.e., cancer, PCOS etc), as factors associated with chronic disease have the potential to impact both our exposure and outcomes. Finally, we excluded participants >35 years, a well-recognized age of fertility decline [234]. Given the small sample size, it was not surprising that the results were unchanged, and believe that these sensitivity analyses or concepts should be addressed in larger studies.

5.1 Strengths and Limitations

Overall, this study has a multitude of strengths. Firstly, the use of fertility treatment and preservation is largely increasing and studies assessing its impact on kidney function are largely lacking making it an important knowledge gap to be addressed. Importantly, this study is the first of its kind assessing the impact of COS on healthy kidney function, utilizing measures including measured GFR and ACR. Further, the translational nature of this study using actual COS cycles

(ie a non-manipulated environment) increases the relevance of the study outcomes to the population of individuals being treated with COS. This study also has important limitations. The sample size of the study is small and thus, compromised the statistical power of our study. Additionally there was marked heterogeneity within our study population with respect to comorbidities, type of infertility, baseline hormone levels, and COS treatment, all of which may have influenced our interpretation of the relationship between COS and kidney function. However, it provided us with baseline information for observing trends. Further, the government of Alberta's current policy states that there is no public funding for COS treatment [235], which may have impacted the diversity of individuals that are accessing COS at the RFP, thereby creating potential for a more homogenous study population with respect to socioeconomic status [236]. However, there already exists a socioeconomic skew in Canada with respect to the population accessing COS treatment, and we also specifically incorporated measures to ensure socioeconomic diversity of participants. Further, this study is not a randomized-controlled trial (RCT), which would provide the highest level of evidence for a single study. However, it would be unethical to randomize participants to be treated with COS/placebo, and through a paired analysis (in which participants acted as their own controls) in this prospective study, we can assess for a potential causative relationship. Finally, there is no long-term follow up of study outcomes beyond COS, but this study provided baseline information that will inform future research studies addressing the long-term effects of COS on kidney health.

CHAPTER SIX: CONCLUSION

In summary, this study demonstrated that COS resulted in no significant change to mGFR and ACR, though a trend towards an increase in mGFR and reduction in ACR was noted. Further, a trend towards a negative relationship between percent change in estrogen level and percent change in ACR was observed. These trends, especially in context of limited power related to a small sample size, indicate that COS has the potential to impact kidney function, as measured by mGFR and ACR.

Future studies should explore the effects of COS on kidney health in a larger sample size with adequate power to further solidify our understanding of this relationship. Further, researchers should also consider assessing other kidney health measures such as kidney plasma flow and filtration fraction to better understand the impact of COS on kidney function. Finally, future studies should address not only the short-term, but also the long-term, effects of COS on kidney health.

Overall, elucidating the influence of COS on kidney function and health will not only improve care for patients accessing fertility treatment and/or preservation, but it will also allow us to further inform females about their reproductive choices and provide knowledge and tools for their care providers.

REFERENCES

1. Vaidya, S.R. and N.R. Aeddula, *Chronic Kidney Disease*, in *StatPearls*. 2024: Treasure Island (FL) with ineligible companies. Disclosure: Narothama Aeddula declares no relevant financial relationships with ineligible companies.
2. *KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease.* Kidney Int, 2024. **105**(4s): p. S117-s314.
3. Webster, A.C., et al., *Chronic Kidney Disease.* Lancet, 2017. **389**(10075): p. 1238-1252.
4. Hill, N.R., et al., *Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis.* PLoS One, 2016. **11**(7): p. e0158765.
5. Meguid El Nahas, A. and A.K. Bello, *Chronic kidney disease: the global challenge.* Lancet, 2005. **365**(9456): p. 331-40.
6. *The global issue of kidney disease.* Lancet, 2013. **382**(9887): p. 101.
7. *Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.* Lancet, 2015. **385**(9963): p. 117-71.
8. Rhee, C.M. and C.P. Kovesdy, *Epidemiology: Spotlight on CKD deaths—increasing mortality worldwide.* Nat Rev Nephrol, 2015. **11**(4): p. 199-200.
9. *The top 10 causes of death*. 2020 [cited 2023 7 April]; Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.
10. Foreman, K.J., et al., *Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories.* Lancet, 2018. **392**(10159): p. 2052-2090.
11. *Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017.* Lancet, 2020. **395**(10225): p. 709-733.
12. Bello, A.K., et al., *Prevalence and Demographics of CKD in Canadian Primary Care Practices: A Cross-sectional Study.* Kidney International Reports, 2019. **4**(4): p. 561-570.
13. Manns, B., et al., *The Financial Impact of Advanced Kidney Disease on Canada Pension Plan and Private Disability Insurance Costs.* Can J Kidney Health Dis, 2017. **4**: p. 2054358117703986.
14. Manns, B.J., D.C. Mendelssohn, and K.J. Taub, *The economics of end-stage renal disease care in Canada: incentives and impact on delivery of care.* Int J Health Care Finance Econ, 2007. **7**(2-3): p. 149-69.
15. Kefale, B., et al., *Quality of life and its predictors among patients with chronic kidney disease: A hospital-based cross sectional study.* PLoS One, 2019. **14**(2): p. e0212184.
16. Pagels, A.A., et al., *Health-related quality of life in different stages of chronic kidney disease and at initiation of dialysis treatment.* Health Qual Life Outcomes, 2012. **10**: p. 71.
17. Hemmelgarn, B.R., et al., *Determining the research priorities for patients with chronic kidney disease not on dialysis.* Nephrol Dial Transplant, 2017. **32**(5): p. 847-854.
18. Mills, K.T., et al., *A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010.* Kidney Int, 2015. **88**(5): p. 950-7.

19. Ricardo, A.C., et al., *Sex-Related Disparities in CKD Progression.* J Am Soc Nephrol, 2019. **30**(1): p. 137-146.
20. Carrero, J.J., et al., *Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease.* Nat Rev Nephrol, 2018. **14**(3): p. 151-164.
21. Antlanger, M., et al., *Sex Differences in Kidney Replacement Therapy Initiation and Maintenance.* Clin J Am Soc Nephrol, 2019. **14**(11): p. 1616-1625.
22. Hodlmoser, S., et al., *Kidney Function, Kidney Replacement Therapy, and Mortality in Men and Women.* Kidney Int Rep, 2022. **7**(3): p. 444-454.
23. Almutary, H., A. Bonner, and C. Douglas, *WHICH PATIENTS WITH CHRONIC KIDNEY DISEASE HAVE THE GREATEST SYMPTOM BURDEN? A COMPARATIVE STUDY OF ADVANCED CKD STAGE AND DIALYSIS MODALITY.* J Ren Care, 2016. **42**(2): p. 73-82.
24. Nitsch, D., et al., *Associations of estimated glomerular filtration rate and albuminuria with mortality and renal failure by sex: a meta-analysis.* BMJ, 2013. **346**: p. f324.
25. Ahmed, S.B. and S.M. Dumanski, *Do Sex and Gender Matter in Kidney and Cardiovascular Disease?* Am J Kidney Dis, 2021. **78**(2): p. 177-179.
26. Dumanski, S.M. and S.B. Ahmed, *Fertility and reproductive care in chronic kidney disease.* J Nephrol, 2019. **32**(1): p. 39-50.
27. Iglesias, P., J.J. Carrero, and J.J. Díez, *Gonadal dysfunction in men with chronic kidney disease: clinical features, prognostic implications and therapeutic options.* J Nephrol, 2012. **25**(1): p. 31-42.
28. Dumanski, S.M., D. Eckersten, and G.B. Piccoli, *Reproductive Health in Chronic Kidney Disease: The Implications of Sex and Gender.* Seminars in Nephrology, 2022. **42**(2): p. 142-152.
29. Burgner, A. and M.A. Hladunewich, *Women's Reproductive Health for the Nephrologist.* American Journal of Kidney Diseases, 2019. **74**(5): p. 675-681.
30. Kuczera, P., A. Wiecek, and M. Adamczak, *Impaired fertility in women and men with chronic kidney disease.* Adv Clin Exp Med, 2022. **31**(2): p. 187-195.
31. Ahmed, S.B. and S. Ramesh, *Sex hormones in women with kidney disease.* Nephrol Dial Transplant, 2016. **31**(11): p. 1787-1795.
32. Piccoli, G.B., et al., *Pregnancy and chronic kidney disease: a challenge in all CKD stages.* Clin J Am Soc Nephrol, 2010. **5**(5): p. 844-55.
33. Edey, M.M., *Male Sexual Dysfunction and Chronic Kidney Disease.* Front Med (Lausanne), 2017. **4**: p. 32.
34. Wiles, K., et al., *Anti-Müllerian hormone concentrations in women with chronic kidney disease.* Clin Kidney J, 2021. **14**(2): p. 537-542.
35. Ramesh, S., et al., *Sex Hormone Status in Women With Chronic Kidney Disease: Survey of Nephrologists' and Renal Allied Health Care Providers' Perceptions.* Can J Kidney Health Dis, 2017. **4**: p. 2054358117734534.
36. Tong, A., et al., *Perspectives on Pregnancy in Women With CKD: A Semistructured Interview Study.* Am J Kidney Dis, 2015. **66**(6): p. 951-61.
37. Piccoli, G.B., et al., *The children of dialysis: live-born babies from on-dialysis mothers in Italy--an epidemiological perspective comparing dialysis, kidney transplantation and the overall population.* Nephrol Dial Transplant, 2014. **29**(8): p. 1578-86.

38. Eun, Y., et al., *Association between female reproductive factors and gout: a nationwide population-based cohort study of 1 million postmenopausal women.* Arthritis research & therapy, 2021. **23**(1): p. 304.
39. Sharma, S.K., et al., *Ovarian dysfunction with moderate-dose intravenous cyclophosphamide (modified NIH regimen) and mycophenolate mofetil in young adults with severe lupus: a prospective cohort study.* Arthritis Research & Therapy, 2020. **22**: p. 1-8.
40. LIM, V.S., et al., *Ovarian function in chronic renal failure: evidence suggesting hypothalamic anovulation.* Annals of internal medicine, 1980. **93**(1_Part_1): p. 21-27.
41. Holley, J.L., *The hypothalamic-pituitary axis in men and women with chronic kidney disease.* Advances in chronic kidney disease, 2004. **11**(4): p. 337-341.
42. Van Eps, C., et al., *Changes in serum prolactin, sex hormones and thyroid function with alternate nightly nocturnal home haemodialysis.* Nephrology, 2012. **17**(1): p. 42-47.
43. Ahmed, S.B., W.S. Vitek, and J.L. Holley, *Fertility, Contraception, and Novel Reproductive Technologies in Chronic Kidney Disease.* Semin Nephrol, 2017. **37**(4): p. 327-336.
44. SIEVERTSEN, G.D., et al., *Metabolic clearance and secretion rates of human prolactin in normal subjects and in patients with chronic renal failure.* The Journal of Clinical Endocrinology & Metabolism, 1980. **50**(5): p. 846-852.
45. Iancu, M.E., A.I. Albu, and D.N. Albu, *Prolactin Relationship with Fertility and In Vitro Fertilization Outcomes—A Review of the Literature.* Pharmaceuticals, 2023. **16**(1): p. 122.
46. Tritos, N.A. and A. Klibanski, *Chapter 3 - Prolactin and Its Role in Human Reproduction*, in *Yen and Jaffe's Reproductive Endocrinology (Eighth Edition)*, J.F. Strauss and R.L. Barbieri, Editors. 2019, Elsevier: Philadelphia. p. 58-74.e8.
47. Wang, G.-c., et al., *Measurements of serum pituitary-gonadal hormones and investigation of sexual and reproductive functions in kidney transplant recipients.* International journal of nephrology, 2010. **2010**.
48. Filocamo, M.T., et al., *Sexual dysfunction in women during dialysis and after renal transplantation.* The journal of sexual medicine, 2009. **6**(11): p. 3125-3131.
49. Dumanski, S.M., et al., *Anti-Müllerian hormone and vascular dysfunction in women with chronic kidney disease.* Physiological Reports, 2022. **10**(2): p. e15154.
50. Stoumpos, S., et al., *The utility of anti-Müllerian hormone in women with chronic kidney disease, on haemodialysis and after kidney transplantation.* Reproductive BioMedicine Online, 2018. **36**(2): p. 219-226.
51. Sikora-Grabka, E., et al., *Serum anti-Müllerian hormone concentration in young women with chronic kidney disease on hemodialysis, and after successful kidney transplantation.* Kidney and Blood Pressure Research, 2016. **41**(5): p. 552-560.
52. Wallace, M.A., *Anatomy and physiology of the kidney.* AORN J, 1998. **68**(5): p. 800, 803-16, 819-20; quiz 821-4.
53. Dalal, R., Z.S. Bruss, and J.S. Sehdev, *Physiology, Renal Blood Flow and Filtration*, in *StatPearls*. 2023: Treasure Island (FL).
54. Soriano, R.M., D. Penfold, and S.W. Leslie, *Anatomy, Abdomen and Pelvis: Kidneys*, in *StatPearls*. 2024: Treasure Island (FL) ineligible companies. Disclosure: Dana Penfold

declares no relevant financial relationships with ineligible companies. Disclosure: Stephen Leslie declares no relevant financial relationships with ineligible companies.
55. Scott, R.P. and S.E. Quaggin, *The cell biology of renal filtration.* Journal of cell biology, 2015. **209**(2): p. 199-210.
56. Bertram, J.F., et al., *Human nephron number: implications for health and disease.* Pediatric Nephrology, 2011. **26**(9): p. 1529-1533.
57. Murawski, I.J., R.W. Maina, and I.R. Gupta, *The relationship between nephron number, kidney size and body weight in two inbred mouse strains.* Organogenesis, 2010. **6**(3): p. 189-94.
58. Madrazo-Ibarra, A. and P. Vaitla, *Histology, Nephron*, in *StatPearls*. 2023: Treasure Island (FL).
59. McCormick, J.A. and D.H. Ellison, *Distal convoluted tubule.* Compr Physiol, 2015. **5**(1): p. 45-98.
60. Brown, A.L., Jr., *The structure of the nephron.* Med Clin North Am, 1966. **50**(4): p. 927-35.
61. Molnar, C. and J. Gair, *22.2. The Kidneys and Osmoregulatory Organs.* Concepts of Biology-1st Canadian Edition, 2013.
62. Kaufman, D.P., H. Basit, and S.J. Knohl, *Physiology, Glomerular Filtration Rate*, in *StatPearls*. 2024, StatPearls Publishing

63. Falkson, S.R. and B. Bordoni, *Anatomy, Abdomen and Pelvis: Bowman Capsule*, in *StatPearls*. 2023: Treasure Island (FL).
64. Ogobuiro, I. and F. Tuma, *Physiology, Renal*, in *StatPearls*. 2024: Treasure Island (FL) with ineligible companies. Disclosure: Faiz Tuma declares no relevant financial relationships with ineligible companies.
65. BagaRao, S. and D.N. Ghista, *Chapter 9 - Renal Physiology Theory and Functional Assessment*, in *Biomedical Engineering of Pancreatic, Pulmonary, and Renal Systems, and Applications to Medicine*, D. Ghista, Editor. 2023, Academic Press. p. 543-572.
66. Wang, X. and M.R. Garrett, *Nephron number, hypertension, and CKD: physiological and genetic insight from humans and animal models.* Physiol Genomics, 2017. **49**(3): p. 180-192.
67. Redal-Baigorri, B., K. Rasmussen, and J.G. Heaf, *Indexing glomerular filtration rate to body surface area: clinical consequences.* J Clin Lab Anal, 2014. **28**(2): p. 83-90.
68. Johnson, R.J., J. Feehally, and J. Floege, *Comprehensive clinical nephrology E-Book*. 2014: Elsevier Health Sciences.
69. Shannon, J.A. and H.W. Smith, *The excretion of inulin, xylose and urea by normal and phlorizinized man.* The Journal of clinical investigation, 1935. **14**(4): p. 393-401.
70. Soveri, I., et al., *Measuring GFR: a systematic review.* Am J Kidney Dis, 2014. **64**(3): p. 411-24.
71. Inker, L.A. and S. Titan, *Measurement and Estimation of GFR for Use in Clinical Practice: Core Curriculum 2021.* Am J Kidney Dis, 2021. **78**(5): p. 736-749.
72. Das, S.K., et al., *Correlation of eGFR By MDRD and CKD-EPI Formula with Creatinine Clearance Estimation in CKD Patients and Healthy Subjects.* Mymensingh Med J, 2021. **30**(1): p. 35-42.

73. White, S.L., et al., *Comparison of the Prevalence and Mortality Risk of CKD in Australia Using the CKD Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) Study GFR Estimating Equations: The AusDiab (Australian Diabetes, Obesity and Lifestyle) Study.* American Journal of Kidney Diseases, 2010. **55**(4): p. 660-670.
74. Inker, L.A., et al., *New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race.* N Engl J Med, 2021. **385**(19): p. 1737-1749.
75. Ebert, N., et al., *Assessment of kidney function: clinical indications for measured GFR.* Clin Kidney J, 2021. **14**(8): p. 1861-1870.
76. Aakhus, T., et al., *Tolerance and excretion of iohexol after intravenous injection in healthy volunteers: Preliminary report.* Acta Radiologica. Diagnosis, 1980. **21**(362_suppl): p. 131-134.
77. Aurell, M., *Accurate and feasible measurements of GFR—is the iohexol clearance the answer?* Nephrology Dialysis Transplantation, 1994. **9**(9): p. 1222-1224.
78. Heron, C., S. Underwood, and P. Dawson, *Electrocardiographic changes during intravenous urography: a study with sodium iothalamate and iohexol.* Clinical radiology, 1984. **35**(2): p. 137-141.
79. Mian, A.N. and G.J. Schwartz, *Chapter 69 - Tests of Kidney Function in Children*, in *Pediatric Critical Care (Fourth Edition)*, B.P. Fuhrman and J.J. Zimmerman, Editors. 2011, Mosby: Saint Louis. p. 998-1008.
80. Brown, S. and P. O'reilly, *Iohexol clearance for the determination of glomerular filtration rate in clinical practice: evidence for a new gold standard.* The Journal of urology, 1991. **146**(3): p. 675-679.
81. Stevens, L.A. and A.S. Levey, *Measured GFR as a confirmatory test for estimated GFR.* Journal of the American society of nephrology, 2009. **20**(11): p. 2305-2313.
82. Delanaye, P., et al., *Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: How to measure glomerular filtration rate with iohexol?* Clin Kidney J, 2016. **9**(5): p. 682-99.
83. Sterner, G., et al., *Determining 'true' glomerular filtration rate in healthy adults using infusion of inulin and comparing it with values obtained using other clearance techniques or prediction equations.* Scand J Urol Nephrol, 2008. **42**(3): p. 278-85.
84. Johnson, D.W., et al., *Chronic kidney disease and measurement of albuminuria or proteinuria: a position statement.* Med J Aust, 2012. **197**(4): p. 224-5.
85. Moman, R.N., N. Gupta, and M. Varacallo, *Physiology, Albumin*, in *StatPearls*. 2023: Treasure Island (FL).
86. Gburek, J., B. Konopska, and K. Gołąb, *Renal Handling of Albumin-From Early Findings to Current Concepts.* Int J Mol Sci, 2021. **22**(11).
87. Keane, W.F. and G. Eknoyan, *Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE): a position paper of the National Kidney Foundation.* Am J Kidney Dis, 1999. **33**(5): p. 1004-10.
88. Yang, C., et al., *The potential of spot and overnight urine in the evaluating of proteinuria and electrolyte.* URINE, 2021. **3**: p. 7-13.
89. Seegmiller, J.C., W.G. Miller, and L.M. Bachmann, *Moving Toward Standardization of Urine Albumin Measurements.* Ejifcc, 2017. **28**(4): p. 258-267.

90. Ralston, S.H., et al., *Screening for proteinuria in a rheumatology clinic: comparison of dipstick testing, 24 hour urine quantitative protein, and protein/creatinine ratio in random urine samples.* Ann Rheum Dis, 1988. **47**(9): p. 759-63.
91. Ginsberg, J.M., et al., *Use of single voided urine samples to estimate quantitative proteinuria.* N Engl J Med, 1983. **309**(25): p. 1543-6.
92. Jensen, M.B., et al., *Quantification of urinary albumin and -creatinine: A comparison study of two analytical methods and their impact on albumin to creatinine ratio.* Clinical Biochemistry, 2022. **108**: p. 5-9.
93. Rao, S., et al., *Accuracy of Spot Urine Albumin Creatinine Ratio With Respect to 24-Hour Urine Albumin for the Detection of Proteinuria in Antenatal Women With Preeclampsia: A Descriptive Study.* Cureus, 2023. **15**(6): p. e39961.
94. Yu, G., et al., *Comparison of 24-h Urine Protein, Urine Albumin-to-Creatinine Ratio, and Protein-to-Creatinine Ratio in IgA Nephropathy.* Front Med (Lausanne), 2022. **9**: p. 809245.
95. Ying, T., et al., *Predictive value of spot versus 24-hour measures of proteinuria for death, end-stage kidney disease or chronic kidney disease progression.* BMC Nephrology, 2018. **19**(1): p. 55.
96. Guy, M., et al., *Protein and albumin-to-creatinine ratios in random urines accurately predict 24 h protein and albumin loss in patients with kidney disease.* Ann Clin Biochem, 2009. **46**(Pt 6): p. 468-76.
97. Inker, L.A., *Albuminuria: time to focus on accuracy.* Am J Kidney Dis, 2014. **63**(3): p. 378-81.
98. Gross, M.L., et al., *Beneficial Effects of Estrogens on Indices of Renal Damage in Uninephrectomized SHRsp Rats.* J Am Soc Nephrol, 2004. **15**(2): p. 348-58.
99. Mercantepe, T., et al., *Protective effects of estrogen and bortezomib in kidney tissue of post-menopausal rats: an ultrastructural study.* Ren Fail, 2016. **38**(7): p. 1129-35.
100. Eriksen, B.O. and O.C. Ingebretsen, *The progression of chronic kidney disease: a 10-year population-based study of the effects of gender and age.* Kidney Int, 2006. **69**(2): p. 375-82.
101. Halbesma, N., et al., *Gender differences in predictors of the decline of renal function in the general population.* Kidney Int, 2008. **74**(4): p. 505-12.
102. Kummer, S., et al., *The influence of gender and sexual hormones on incidence and outcome of chronic kidney disease.* Pediatr Nephrol, 2012. **27**(8): p. 1213-9.
103. Kattah, A.G., et al., *CKD in Patients with Bilateral Oophorectomy.* Clin J Am Soc Nephrol, 2018. **13**(11): p. 1649-1658.
104. Chapman, A.B., et al., *Systemic and renal hemodynamic changes in the luteal phase of the menstrual cycle mimic early pregnancy.* Am J Physiol, 1997. **273**(5): p. F777-82.
105. Farahmand, M., et al., *Endogenous estrogen exposure and chronic kidney disease; a 15-year prospective cohort study.* BMC Endocrine Disorders, 2021. **21**: p. 1-8.
106. Zhang, K., et al., *Follicle-stimulating hormone promotes renal tubulointerstitial fibrosis in aging women via the AKT/GSK-3β/β-catenin pathway.* Aging Cell, 2019. **18**(5): p. e12997.
107. Li, Q., et al., *High Circulating Follicle-Stimulating Hormone Level Is a Potential Risk Factor for Renal Dysfunction in Post-Menopausal Women.* Front Endocrinol (Lausanne), 2021. **12**: p. 627903.

108. Zhou, Y., et al., *Elevated circulating luteinizing hormone levels are associated with diabetic macroalbuminuria in Chinese men and postmenopausal women: a cross-sectional study.* Journal of Diabetes, 2020. **12**(11): p. 819-833.
109. Economic, U.N.D.o. and S.A.P. Division, *World Contraceptive Use*. 2019: United Nations.
110. Armstrong, C., *ACOG guidelines on noncontraceptive uses of hormonal contraceptives.* American Family Physician, 2010. **82**(3): p. 288-295.
111. Cooper, D.B., P. Patel, and H. Mahdy, *Oral Contraceptive Pills*, in *StatPearls*. 2024: Treasure Island (FL) ineligible companies. Disclosure: Preeti Patel declares no relevant financial relationships with ineligible companies. Disclosure: Heba Mahdy declares no relevant financial relationships with ineligible companies.
112. Keating, N.L., et al., *Use of hormone replacement therapy by postmenopausal women in the United States.* Annals of internal medicine, 1999. **130**(7): p. 545-553.
113. Bassuk, S.S. and J.E. Manson, *Oral contraceptives and menopausal hormone therapy: relative and attributable risks of cardiovascular disease, cancer, and other health outcomes.* Ann Epidemiol, 2015. **25**(3): p. 193-200.
114. Ahmed, S.B., et al., *Oral contraceptives, angiotensin-dependent renal vasoconstriction, and risk of diabetic nephropathy.* Diabetes care, 2005. **28**(8): p. 1988-1994.
115. Ribstein, J., et al., *Renal characteristics and effect of angiotensin suppression in oral contraceptive users.* Hypertension, 1999. **33**(1): p. 90-95.
116. Monster, T.B., et al., *Oral contraceptive use and hormone replacement therapy are associated with microalbuminuria.* Arch Intern Med, 2001. **161**(16): p. 2000-5.
117. Al-Jomard, S.A. and W.B. Al-Youzbaki, *Effect of combined oral contraceptive pills on renal function tests.* Iraqi J Comm Med, 2012. **4**: p. 314-319.
118. Atthobari, J., et al., *The impact of hormonal contraceptives on blood pressure, urinary albumin excretion and glomerular filtration rate.* Br J Clin Pharmacol, 2007. **63**(2): p. 224-31.
119. Chang, D.H., Riehl-Tonn, V.J., Ronksley, E.P., Ahmed, S.B., Ganshorn, H., Brennand, E.A., Dumanski, S.M., *Effects of Hormonal Contraception on Kidney Function in Premenopausal Females: A Systematic Review and Meta-Analysis.* Unpublished Data, 2024.
120. Ahmed, S.B., et al., *Oral estrogen therapy in postmenopausal women is associated with loss of kidney function.* Kidney Int, 2008. **74**(3): p. 370-6.
121. Kaygusuz, I., et al., *Does hormone replacement therapy have beneficial effects on renal functions in menopausal women?* Arch Gynecol Obstet, 2012. **285**(6): p. 1643-6.
122. Stachenfeld, N.S. and H.S. Taylor, *Progesterone increases plasma volume independent of estradiol.* Journal of applied physiology, 2005. **98**(6): p. 1991-1997.
123. Stachenfeld, N.S. and H.S. Taylor, *Effects of estrogen and progesterone administration on extracellular fluid.* Journal of Applied Physiology, 2004. **96**(3): p. 1011-1018.
124. Sarna, M.A., et al., *Oral contraceptive progestins and angiotensin-dependent control of the renal circulation in humans.* J Hum Hypertens, 2009. **23**(6): p. 407-14.
125. Hembree, W.C., et al., *Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline.* The Journal of Clinical Endocrinology & Metabolism, 2017. **102**(11): p. 3869-3903.

126. Coleman, E., et al., *Standards of Care for the Health of Transgender and Gender Diverse People, Version 8.* Int J Transgend Health, 2022. **23**(Suppl 1): p. S1-s259.
127. Spanos, C., et al., *Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: a systematic review.* World journal of diabetes, 2020. **11**(3): p. 66.
128. Klaver, M., et al., *Cross-sex hormone therapy in transgender persons affects total body weight, body fat and lean body mass: a meta-analysis.* Andrologia, 2017. **49**(5): p. e12660.
129. Giltay, E., et al., *Effects of sex steroids on plasma total homocysteine levels: a study in transsexual males and females.* The Journal of Clinical Endocrinology & Metabolism, 1998. **83**(2): p. 550-553.
130. Krupka, E., et al., *The Effect of Gender-Affirming Hormone Therapy on Measures of Kidney Function: A Systematic Review and Meta-Analysis.* Clin J Am Soc Nephrol, 2022. **17**(9): p. 1305-1315.
131. Eckenrode, H.E., J.C. Carwie, and L.M. Curtis. *Does Gender Affirming Hormone Therapy Increase the Risk of Kidney Disease?* in *Seminars in Nephrology*. 2022. Elsevier.
132. Collister, D., et al., *Providing care for transgender persons with kidney disease: a narrative review.* Canadian Journal of Kidney Health and Disease, 2021. **8**: p. 2054358120985379.
133. Vander Borght, M. and C. Wyns, *Fertility and infertility: Definition and epidemiology.* Clin Biochem, 2018. **62**: p. 2-10.
134. Lo, W. and L. Campo-Engelstein. *Expanding the Clinical Definition of Infertility to Include Socially Infertile Individuals and Couples*. in *Reproductive Ethics II*. 2018. Cham: Springer International Publishing.
135. Barbieri, R.L., *Chapter 22 - Female Infertility*, in *Yen and Jaffe's Reproductive Endocrinology (Eighth Edition)*, J.F. Strauss and R.L. Barbieri, Editors. 2019, Elsevier: Philadelphia. p. 556-581.e7.
136. Matzuk, M.M. and D.J. Lamb, *The biology of infertility: research advances and clinical challenges.* Nat Med, 2008. **14**(11): p. 1197-213.
137. Thonneau, P., et al., *Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989).* Hum Reprod, 1991. **6**(6): p. 811-6.
138. Thoma, M., et al., *Biological and Social Aspects of Human Infertility: A Global Perspective*. 2021, Oxford University Press.
139. Van Balen, F. and T. Gerrits, *Quality of infertility care in poor-resource areas and the introduction of new reproductive technologies.* Human Reproduction, 2001. **16**(2): p. 215-219.
140. Dyer, S.J., *The value of children in African countries–insights from studies on infertility.* Journal of Psychosomatic Obstetrics & Gynecology, 2007. **28**(2): p. 69-77.
141. Van Balen, F. and H.M. Bos, *The social and cultural consequences of being childless in poor-resource areas.* Facts, views & vision in ObGyn, 2009. **1**(2): p. 106.
142. Gerrits, T. and M. Shaw, *Biomedical infertility care in sub-Saharan Africa: a social science review of current practices, experiences and view points.* Facts, views & vision in ObGyn, 2010. **2**(3): p. 194.

143. Inhorn, M.C. and P. Patrizio, *Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century.* Human reproduction update, 2015. **21**(4): p. 411-426.
144. Shreffler, K.M., A.L. Greil, and J. McQuillan, *Responding to infertility: Lessons from a growing body of research and suggested guidelines for practice.* Family relations, 2017. **66**(4): p. 644-658.
145. Katz, P., et al., *Costs of infertility treatment: results from an 18-month prospective cohort study.* Fertil Steril, 2011. **95**(3): p. 915-21.
146. Chambers, G.M., et al., *The economic impact of assisted reproductive technology: a review of selected developed countries.* Fertility and Sterility, 2009. **91**(6): p. 2281-2294.
147. Adewunmi, A.A., et al., *Factors associated with acceptability of child adoption as a management option for infertility among women in a developing country.* Int J Womens Health, 2012. **4**: p. 365-72.
148. Brinsden, P.R., *Gestational surrogacy.* Hum Reprod Update, 2003. **9**(5): p. 483-91.
149. Jain, M. and M. Singh, *Assisted Reproductive Technology (ART) Techniques*, in *StatPearls*. 2023: Treasure Island (FL).
150. Sunderam, S., et al., *Assisted Reproductive Technology Surveillance - United States, 2016.* MMWR Surveill Summ, 2019. **68**(4): p. 1-23.
151. Kissin, D.M., et al., *Fertility Treatments in the United States: Improving Access and Outcomes.* Obstet Gynecol, 2016. **128**(2): p. 387-390.
152. Allahbadia, G.N., *Intrauterine Insemination: Fundamentals Revisited.* J Obstet Gynaecol India, 2017. **67**(6): p. 385-392.
153. *Canadian Assisted Reproductive Technologies Register Plus (CARTR Plus)*, in *Canadian Fertility and Andrology Society - 65th Annual Meeting*, A. Lanes, et al., Editors. 2019, Better Outcomes & Network Ontario: Ottawa, Canada. p. 97.
154. Gunby, J., et al., *Assisted reproductive technologies (ART) in Canada: 2006 results from the Canadian ART Register.* Fertil Steril, 2010. **93**(7): p. 2189-201.
155. *Births, 2017*. [Website] 2018 [cited 2023 April 7].
156. Choe, J. and A.L. Shanks, *In Vitro Fertilization*, in *StatPearls*. 2023: Treasure Island (FL).
157. Howie, R. and V. Kay, *Controlled ovarian stimulation for in-vitro fertilization.* Br J Hosp Med (Lond), 2018. **79**(4): p. 194-199.
158. Depalo, R., et al., *GnRH agonist versus GnRH antagonist in in vitro fertilization and embryo transfer (IVF/ET).* Reprod Biol Endocrinol, 2012. **10**: p. 26.
159. Barbieri, E., S. Kashyap, and P.H. Chung, *Chapter 35 - Infertility and In Vitro Fertilization*, in *Principles of Gender-Specific Medicine (Second Edition)*, M.J. Legato, Editor. 2010, Academic Press: San Diego. p. 381-399.
160. Medicine, E.C.o.t.A.S.f.R., *Fertility preservation and reproduction in patients facing gonadotoxic therapies: a committee opinion.* Fertility and Sterility, 2013. **100**(5): p. 1224-1231.
161. Donnez, J. and M.-M. Dolmans, *Fertility preservation in women.* Nature Reviews Endocrinology, 2013. **9**(12): p. 735-749.
162. Gonzalez, C., et al., *Concise review: fertility preservation: an update.* Stem Cells Transl Med, 2012. **1**(9): p. 668-72.

163. Bedoschi, G. and K. Oktay, *Current approach to fertility preservation by embryo cryopreservation.* Fertility and sterility, 2013. **99**(6): p. 1496-1502.
164. Argyle, C.E., J.C. Harper, and M.C. Davies, *Oocyte cryopreservation: where are we now?* Hum Reprod Update, 2016. **22**(4): p. 440-9.
165. Roque, M., et al., *Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis.* Fertility and sterility, 2013. **99**(1): p. 156-162.
166. Arav, A., et al., *New methods for cooling and storing oocytes and embryos in a clean environment of -196 degrees C.* Reprod Biomed Online, 2016. **33**(1): p. 71-8.
167. Jain, J.K. and R.J. Paulson, *Oocyte cryopreservation.* Fertility and Sterility, 2006. **86**(4, Supplement): p. 1037-1046.
168. Van Voorhis, B.J., et al., *What do consistently high-performing in vitro fertilization programs in the US do?* Fertility and sterility, 2010. **94**(4): p. 1346-1349.
169. La Marca, A. and S.K. Sunkara, *Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice.* Human reproduction update, 2014. **20**(1): p. 124-140.
170. Jungheim, E.S., M.F. Meyer, and D.E. Broughton. *Best practices for controlled ovarian stimulation in in vitro fertilization*. in *Seminars in reproductive medicine*. 2015. Thieme Medical Publishers.
171. Nardo, L., et al., *Controlled ovarian hyperstimulation regimens: a review of the available evidence for clinical practice. Produced on behalf of the BFS Policy and Practice Committee.* Human Fertility, 2013. **16**(3): p. 144-150.
172. Depalo, R., et al., *GnRH agonist versus GnRH antagonist in in vitro fertilization and embryo transfer (IVF/ET).* Reproductive Biology and Endocrinology, 2012. **10**(1): p. 26.
173. Grow, D., et al., *GnRH agonist and GnRH antagonist protocols: comparison of outcomes among good-prognosis patients using national surveillance data.* Reproductive biomedicine online, 2014. **29**(3): p. 299-304.
174. Shrestha, D., X. La, and H.L. Feng, *Comparison of different stimulation protocols used in in vitro fertilization: a review.* Ann Transl Med, 2015. **3**(10): p. 137.
175. Kolibianakis, E.M., et al., *Effect of oral contraceptive pill pretreatment on ongoing pregnancy rates in patients stimulated with GnRH antagonists and recombinant FSH for IVF. A randomized controlled trial.* Human Reproduction, 2005. **21**(2): p. 352-357.
176. Ghasemzadeh, A., et al., *Effect of Estrogen Priming in Antagonist Cycles in Women With Poor Response to IVF Treatment.* Crescent Journal of Medical & Biological Sciences, 2020. **7**(1).
177. Prasad, S., et al., *Estradiol Level on Day 2 and Day of Trigger: A Potential Predictor of the IVF-ET Success.* J Obstet Gynaecol India, 2014. **64**(3): p. 202-7.
178. Bhandari, H.M., M.K. Choudhary, and J.A. Stewart, *Complications of assisted reproductive technology treatment and the factors influencing reproductive outcome.* The Obstetrician & Gynaecologist, 2018. **20**(3): p. 177-186.
179. Pellicer, N., D. Galliano, and A. Pellicer, *Chapter 22 - Ovarian Hyperstimulation Syndrome*, in *The Ovary (Third Edition)*, P.C.K. Leung and E.Y. Adashi, Editors. 2019, Academic Press. p. 345-362.

180. Kumar, P., et al., *Ovarian hyperstimulation syndrome.* J Hum Reprod Sci, 2011. **4**(2): p. 70-5.
181. Michaelson-Cohen, R., et al., *Does elevated human chorionic gonadotropin alone trigger spontaneous ovarian hyperstimulation syndrome?* Fertility and sterility, 2008. **90**(5): p. 1869-1874.
182. Binder, H., et al., *Update on ovarian hyperstimulation syndrome: Part 1--Incidence and pathogenesis.* Int J Fertil Womens Med, 2007. **52**(1): p. 11-26.
183. Luke, B., *Pregnancy and birth outcomes in couples with infertility with and without assisted reproductive technology: with an emphasis on US population-based studies.* Am J Obstet Gynecol, 2017. **217**(3): p. 270-281.
184. Kulkarni, A.D., et al., *Fertility treatments and multiple births in the United States.* N Engl J Med, 2013. **369**(23): p. 2218-25.
185. Zhu, L., et al., *Maternal and Live-birth Outcomes of Pregnancies following Assisted Reproductive Technology: A Retrospective Cohort Study.* Sci Rep, 2016. **6**: p. 35141.
186. Pandey, S., et al., *Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis.* Hum Reprod Update, 2012. **18**(5): p. 485-503.
187. Ahmed, S.B., et al., *Effects of oral contraceptive use on the renal and systemic vascular response to angiotensin II infusion.* J Am Soc Nephrol, 2004. **15**(3): p. 780-6.
188. Krukowski, R.A. and K.M. Ross, *Measuring Weight with Electronic Scales in Clinical and Research Settings During the Coronavirus Disease 2019 Pandemic.* Obesity (Silver Spring), 2020. **28**(7): p. 1182-1183.
189. Oniszczenko, W. and E. Stanisławiak, *Association between sex and body mass index as mediated by temperament in a nonclinical adult sample.* Eat Weight Disord, 2019. **24**(2): p. 291-298.
190. National Heart, L. and B. Institute, *The practical guide: identification, evaluation, and treatment of overweight and obesity in adults.* Bethesda, MD: National Institutes of Health, 2000. **94**.
191. Ness-Abramof, R. and C.M. Apovian, *Waist circumference measurement in clinical practice.* Nutrition in clinical practice, 2008. **23**(4): p. 397-404.
192. Rabi, D.M., et al., *Hypertension Canada's 2020 comprehensive guidelines for the prevention, diagnosis, risk assessment, and treatment of hypertension in adults and children.* Canadian Journal of Cardiology, 2020. **36**(5): p. 596-624.
193. Bryant, C., J. Moore, and T.E. Curry, *Determination of Serum Estradiol Levels by Radiometric and Chemiluminescent Techniques*, in *Molecular Endocrinology: Methods and Protocols*, O.-K. Park-Sarge and T.E. Curry, Editors. 2009, Humana Press: Totowa, NJ. p. 21-32.
194. Gounden, V., H. Bhatt, and I. Jialal, *Renal Function Tests*, in *StatPearls*. 2023: Treasure Island (FL).
195. Miller, W.G., et al., *Current issues in measurement and reporting of urinary albumin excretion.* Clinical chemistry, 2009. **55**(1): p. 24-38.
196. Chagnac, A. and A.N. Friedman, *Measuring Albuminuria in Individuals With Obesity: Pitfalls of the Urinary Albumin-Creatinine Ratio.* Kidney Medicine, 2024. **6**(4): p. 100804.

197. Sathiananthamoorthy, S., et al., *Reassessment of Routine Midstream Culture in Diagnosis of Urinary Tract Infection.* Journal of Clinical Microbiology, 2019. **57**(3): p. 10.1128/jcm.01452-18.
198. Yuan, H.C., et al., *Alcohol intake and the risk of chronic kidney disease: results from a systematic review and dose–response meta-analysis.* European Journal of Clinical Nutrition, 2021. **75**(11): p. 1555-1567.
199. Briganti, E.M., et al., *Smoking is associated with renal impairment and proteinuria in the normal population: The AusDiab kidney study.* American Journal of Kidney Diseases, 2002. **40**(4): p. 704-712.
200. Giontella, A., et al., *Caffeine Intake, Plasma Caffeine Level, and Kidney Function: A Mendelian Randomization Study.* Nutrients, 2023. **15**(20): p. 4422.
201. Park, F., et al., *Cannabinoids and the kidney: effects in health and disease.* American Journal of Physiology-Renal Physiology, 2017. **313**(5): p. F1124-F1132.
202. Bundy, J.D., et al., *Self-reported tobacco, alcohol, and illicit drug use and progression of chronic kidney disease.* Clinical journal of the American Society of Nephrology, 2018. **13**(7): p. 993-1001.
203. BERGSTRÖM, J., M. AHLBERG, and A. ALVESTRAND, *Influence of protein intake on renal hemodynamics and plasma hormone concentrations in normal subjects.* Acta Medica Scandinavica, 1985. **217**(2): p. 189-196.
204. Heidari, S., et al., *Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use.* Research Integrity and Peer Review, 2016. **1**(1): p. 2.
205. Fung, M.M., et al., *A cross-sectional and 10-year prospective study of postmenopausal estrogen therapy and blood pressure, renal function, and albuminuria: the Rancho Bernardo Study.* Menopause, 2011. **18**(6): p. 629-37.
206. Schopick, E.L., et al., *Post-menopausal hormone use and albuminuria.* Nephrology Dialysis Transplantation, 2009. **24**(12): p. 3739-3744.
207. Brandle, E., et al., *Influence of oral contraceptive agents on kidney function and protein metabolism.* Eur J Clin Pharmacol, 1992. **43**(6): p. 643-6.
208. Agarwal, M., et al., *The relationship between albuminuria and hormone therapy in postmenopausal women.* American journal of kidney diseases, 2005. **45**(6): p. 1019-1025.
209. Szekacs, B., et al., *Postmenopausal hormone replacement improves proteinuria and impaired creatinine clearance in type 2 diabetes mellitus and hypertension.* Bjog, 2000. **107**(8): p. 1017-21.
210. Manning, P.J., et al., *HRT does not improve urinary albumin excretion in postmenopausal diabetic women.* Diabetes research and clinical practice, 2003. **60**(1): p. 33-39.
211. Ekinci, E.I., et al., *Is hyperfiltration associated with higher urine albumin-to-creatinine ratio at follow up among Indigenous Australians? The eGFR follow-up study.* Journal of diabetes and its complications, 2019. **33**(5): p. 343-349.
212. Melsom, T., et al., *Association of increasing GFR with change in albuminuria in the general population.* Clinical Journal of the American Society of Nephrology, 2016. **11**(12): p. 2186-2194.

213. Melsom, T., et al., *Prediabetes and Risk of Glomerular Hyperfiltration and Albuminuria in the General Nondiabetic Population: A Prospective Cohort Study.* American Journal of Kidney Diseases, 2016. **67**(6): p. 841-850.
214. Cachat, F., et al., *A systematic review of glomerular hyperfiltration assessment and definition in the medical literature.* Clinical journal of the American Society of Nephrology, 2015. **10**(3): p. 382-389.
215. Chapman, A.B., et al., *Temporal relationships between hormonal and hemodynamic changes in early human pregnancy.* Kidney international, 1998. **54**(6): p. 2056-2063.
216. Conrad, K.P., *Mechanisms of renal vasodilation and hyperfiltration during pregnancy.* Journal of the Society for Gynecologic Investigation, 2004. **11**(7): p. 438-448.
217. Conrad, K.P., et al., *Role of relaxin in maternal renal vasodilation of pregnancy.* Annals of the New York Academy of Sciences, 2005. **1041**(1): p. 147-154.
218. Christy, N.P. and J.C. Shaver, *Estrogens and the kidney.* Kidney International, 1974. **6**(5): p. 366-376.
219. Hillege, H.L., et al., *Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population.* Circulation, 2002. **106**(14): p. 1777-1782.
220. Wachtell, K., et al., *Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study.* Annals of internal medicine, 2003. **139**(11): p. 901-906.
221. Ninomiya, T., et al., *Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes.* Journal of the American Society of Nephrology, 2009. **20**(8): p. 1813-1821.
222. Heerspink, H.L., et al., *Is a reduction in albuminuria associated with renal and cardiovascular protection? A post hoc analysis of the ALTITUDE trial.* Diabetes, Obesity and Metabolism, 2016. **18**(2): p. 169-177.
223. Forman, J.P. and B.M. Brenner, *'Hypertension' and 'microalbuminuria': the bell tolls for thee.* Kidney Int, 2006. **69**(1): p. 22-8.
224. Orlowski, M. and M.S. Sarao, *Physiology, Follicle Stimulating Hormone*, in *StatPearls*. 2024: Treasure Island (FL) ineligible companies. Disclosure: Manbeer Sarao declares no relevant financial relationships with ineligible companies.
225. Hofmann, G.E., et al., *High-dose follicle-stimulating hormone (FSH) ovarian stimulation in low-responder patients for in vitro fertilization.* J In Vitro Fert Embryo Transf, 1989. **6**(5): p. 285-9.
226. Zhong, Y.-p., et al., *Impact of transitory hyperprolactinemia on clinical outcome of in vitro fertilization and embryo transfer.* Journal of medical biochemistry, 2012. **31**(1): p. 27-33.
227. Crosignani, P., et al., *Human prolactin release induced by follicle stimulating hormone, luteinizing hormone and human chorionic gonadotrophin.* Human Reproduction, 1991. **6**(8): p. 1070-1073.
228. Kamel, A., et al., *Changes in serum prolactin level during intracytoplasmic sperm injection, and effect on clinical pregnancy rate: a prospective observational study.* BMC Pregnancy and Childbirth, 2018. **18**: p. 1-7.

229. Harlow, C., et al., *Stress and stress-related hormones during in-vitro fertilization treatment.* Human Reproduction, 1996. **11**(2): p. 274-279.
230. Bajaj, S.S., B. Jain, and F.C. Stanford, *Weight gain after in vitro fertilization: a potential consequence of controlled ovarian stimulation.* Journal of Assisted Reproduction and Genetics, 2022. **39**(4): p. 973-976.
231. Tso, L.O., et al., *Does the controlled ovarian stimulation increase the weight of women undergoing IVF treatment?* European Journal of Obstetrics & Gynecology and Reproductive Biology, 2021. **263**: p. 205-209.
232. Chavarro, J.E., et al., *Body mass index and short-term weight change in relation to treatment outcomes in women undergoing assisted reproduction.* Fertility and sterility, 2012. **98**(1): p. 109-116.
233. Nguekeu, M.R., et al., *Ovarian Stimulation, Body Image and Suffering: A Case Study of a Cameroonian Woman Facing the Assisted Reproductive Technology.* Psychology, 2022. **13**(6): p. 936-950.
234. Group, E.C.W., *Fertility and ageing.* Human reproduction update, 2005. **11**(3): p. 261-276.
235. Vaidya, A., et al., *Assisted Reproductive Technologies in Alberta: An Economic Analysis to Inform Policy Decision-Making.* Journal of Obstetrics and Gynaecology Canada, 2015. **37**(12): p. 1122-1130.
236. Imrie, R., et al., *Socioeconomic status and fertility treatment outcomes in high-income countries: a review of the current literature.* Human Fertility, 2021: p. 1-11.