Ovulation-Inducing Fertility Treatments and the Risk of Breast Cancer

Adriano Petrangelo

Department of Medicine

Division of Experimental Medicine

McGill University

Montréal, Québec, Canada

April 2019

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Experimental Medicine

Table of Contents

Abstract

Background and Objectives: The interplay between reproductive hormones and breast cancer is well established. The safety of fertility treatments which stimulate follicular development has been questioned as these therapies augment endogenous estrogen to supraphysiologic levels. Two prominent methods of ovarian stimulation are clomiphene and exogenous gonadotropins. The main goal of this manuscript was to evaluate the association between ovarian stimulation and breast cancer. First, a systematic review and meta-analysis of the existing literature on this topic was conducted. Second, an observational study using a case-control design was performed using the Clinical Practice Research Datalink (CPRD).

Materials and Methods: The systematic review of the literature involved searching the MEDLINE and EMBED databases to identify observational studies examining the relationship of interest. Effect measures for studies compliant with inclusion and exclusion criteria were extracted. The selected studies were stratified based on their study design and the type(s) of exposure assessed. Pooling of study data was conducted in accordance with the DerSimonian and Laird method. The case-control study involved identifying cases of breast cancer recorded in the CPRD between 1995 and 2015. Each case of breast cancer was matched to 10 controls based on age, general practice, and length of follow-up within the CPRD. Risk ratios were computed using multivariate logistic regression. The regression model was adjusted for body mass index, smoking, alcohol use, parity, hormonal contraceptive use, hormone replacement therapy use, and oophorectomy. Exposure to either clomiphene or in vitro fertilization (IVF) was assessed for both case and control subjects. The primary analysis characterized exposure on an "ever/never" basis further stratifying for the age of breast cancer diagnosis. Two secondary analyses were performed. The first involved evaluating the dose-response relationship between clomiphene and

breast cancer. The second involved examining whether the results of the primary analysis were confounded by the underlying effects of infertility. This involved stratifying the study population on previous diagnoses of polycystic ovary syndrome (PCOS).

Results: The meta-analysis included 17 cohort studies and 4 case-control studies. Pooled RRs found no association between ovarian stimulation and breast cancer, regardless of the study design or the type of exposure. A secondary analysis stratifying on subject follow-up time showed that breast cancer risk in patients having undergone ovarian stimulation did not increase even with extended periods of follow-up. The case-control study showed conflicting results. Exposure to clomiphene (RR 1.32, 95% CI [1.23-1.42]) and to IVF (RR 1.55, 95% CI [1.42-1.69]) was associated with an increased risk of breast cancer, following adjustment. After stratification for age of diagnosis, IVF remained significantly associated with both pre- and post-menopausal malignancies whereas clomiphene remained solely associated with pre-menopausal breast cancers. The association between clomiphene and breast cancer was not significant in the population of women previously diagnosed with PCOS (1.22 [0.99-1.50]), indicating the results of the primary analysis may have been confounded by the underlying effects of infertility.

Conclusion: Presently, there is no definitive link between ovarian stimulation and breast cancer. While the results of the case-control study indicated the association was significant, the strong possibility that infertility and its associated conditions confounded results suggests that these findings should be interpreted with caution. Furthermore, the existing literature refutes this association. Nevertheless, continued monitoring of this relationship is warranted given its biological plausibility and the increasing use of these treatments across the developed world.

Résumé

Contexte et objectifs : Il existe une relation bien établie entre le taux d'hormones reproductrices et le cancer du sein. L'innocuité des traitements de fertilité qui stimulent le développement folliculaire au sein des ovaires, engendrant une hausse supra-physiologique des taux d'œstrogènes endogènes, est remise en cause. L'administration de clomifène et de gonadotrophines exogènes en sont les deux formes de traitement les plus repandues. L'objectif principal de cette recherche était d'évaluer le lien entre ces traitements de fertilité et le cancer du sein. Dans un premier temps, une étude systématique et une méta-analyse des études sur ce sujet ont été menées. Dans un second temps, une étude observationnelle se basant sur une conception d'étude cas-témoins a été effectuée à l'aide du *Clinical Practice Research Datalink* (CPRD).

Documentations et méthodes : Les bases de données MEDLINE et EMBED ont été explorées afin d'identifier les études observationnelles traitant du sujet. Les résultats des études conformes aux critères d'inclusion et d'exclusion ont été soutirés. Les études sélectionnées ont été stratifiées en se basant sur le modèle d'étude et le type d'exposition évalués. Le regroupement des données des études a été mené conformément à la méthode de DerSimonian et Laird. L'étude cas-témoins a nécessité l'identification des cas de cancer du sein enregistrés dans le CPRD entre 1995 et 2015. Dix cas-témoins ont été appariés à chaque cas de cancer du sein en fonction de l'ancienneté et de la durée du suivi au sein du CPRD. Les taux de risques ont été calculés en utilisant une régression logistique multivariée, prenant en compte l'indice de masse corporelle, le tabagisme, la consommation d'alcool, la parité, l'utilisation de contraceptifs hormonaux, la thérapie de substitution hormonale, et l'ovariectomie. Les cas et les témoins ont été évalués en fonction de leur exposition soit au clomifène soit à la FIV. L'analyse primaire a caractérisé l'exposition sur la base du critère « a été exposé/n'a jamais été exposé », la stratifiant ensuite en fonction de l'ancienneté du diagnostic du cancer du sein. Deux analyses secondaires ont été

réalisées. La première a consisté à apprécier la relation dose-réponse entre le clomifène et le cancer du sein. La deuxième a consisté à évaluer si les résultats de l'analyse primaire ont pu être faussés par les effets de l'infertilité sous-jacente en stratifiant la population étudiée sur des diagnostics de syndrome des ovaires polykystiques (SOPK).

Résultats : La méta-analyse a inclus 17 études de cohortes et 4 cas-témoins. Le regroupement des RR n'a montré aucune corrélation entre stimulation ovarienne et cancer du sein, quels que soient le modèle d'étude et le type d'exposition évalués. Une analyse secondaire après stratification sur la durée du suivi n'a révélé aucun lien significatif. Les études cas-témoins ont démontré des résultats conflictuels. L'exposition au clomifène (RR 1.32, 95% IC [1.23-1.42]) ou à la FIV (RR 1.55, 95% IC [1.42-1.69]) était associée à un risque accru de cancer du sein après ajustement. Après une stratification par ancienneté du diagnostic, la FIV est restée significativement associée aux tumeurs malignes aussi bien post- que pré- ménopause tandis que le clomifène est resté associé seulement aux cancers du sein postménopause. Le lien entre clomifène et cancer du sein n'était pas significatif dans la population des femmes préalablement diagnostiquées avec un SOPK (1.22 [0.99-1.50]), indiquant que les résultats de l'analyse primaire ont pu être faussés par les effets sous-jacents à l'infertilité.

Conclusion : Actuellement, il n'existe pas de lien établi entre la stimulation ovarienne et le cancer du sein. Les résultats de l'étude cas-témoins ont démontré une association possible significative, cependant la forte possibilité que les effets de l'infertilité aient confondu les résultats nécessite d'interpréter ces résultats avec prudence. En outre, la littérature existante réfute généralement cette association. Néanmoins, une surveillance continue de ce lien s'impose compte tenu de sa plausibilité biologique et de la popularité croissante de ces traitements.

Contribution of Authors

Adriano Petrangelo is the primary author of this thesis under the supervision and guidance of Dr. Haim A. Abenhaim.

The study concept and design with planned by both Adriano Petrangelo and Dr. Haim A. Abenhaim, along with the advice of members of the thesis committee, which included Dr. Laurent Azoulay and Dr. Togas Tulandi.

Adriano Petrangelo conducted all analyses for the meta-analysis. The manuscript for the systematic review and meta-analysis was drafted by Adriano Petrangelo.

Analyses for the case-control study were performed through the joint effort of Adriano Petrangelo and Nicholas Czuzoj-Shulman. Adriano Petrangelo drafted the manuscript to this study. Adriano Petrangelo also drafted this study's protocol, which was reviewed and approved by the Independent Scientific Advisory Committee, the regulatory body granting access to the Clinical Practice Research Datalink.

All other sections of the thesis, including the Literature Review, Discussion, Conclusion, and Frameworks for Future Research were authored by Adriano Petrangelo.

The translation of this work's abstract was performed by Adriano Petrangelo with editorial help from Louise Bouchart.

Dr. Haim A. Abenhaim, Dr. Andrea Spence, and Dr. Jacques Balayla reviewed and provided suggestions for all aspects of the thesis.

List of Abbreviations

AMH - Anti-Mullerian Hormone

ART – Assisted Reproductive Technology

ASIR – Age-standardized incidence rate

BMI – Body mass index

cAMP – Cyclic AMP

CPRD – Clinical Practice Research Datalink

EO – Early-onset

ER – Estrogen receptor

FSH – Follicle stimulating hormone

GnRH – Gonadotropin-releasing hormone

HH – Hypogonadotropic hypogonadism

HPG – Hypothalamic-pituitary-gonadal

HR – Hazard ratio

HRT – Hormone replacement therapy

ISAC – Independent Scientific Advisory Committee

IVF – In vitro fertilization

LH – Luteinizing hormone

LO – Late-onset

MCMC – Markov chain Monte Carlo

NHS – National Health Service

NICE – National Institute for Health and Care Excellence

OHSS – Ovarian hyperstimulation syndrome

OR – Odds ratio

PCOS – Polycystic ovary syndrome

POP – Progestin-only pill

PR – Progesterone receptor

RR – Risk ratio

SERM – Selective estrogen receptor modulator

SES – Socioeconomic status

SIR – Standardized incidence ratio

TEB – Terminal end bud

UK – United Kingdom

WHO – World Health Organization

List of Tables and Figures

Appendix

Table A.1: Codes used to identify exposure to clomiphene within the CPRD

Table A.2: Codes used to identify exposure to IVF within the CPRD

Table A.3: Codes used to identify diagnoses of breast cancer within the CPRD

Table A.4: BMI categories

Table A.5: Codes used to evaluate smoking status within the CPRD

Table A.6: Codes used to evaluate alcohol use within the CPRD

Table A.7: Codes used to evaluate parity status within the CPRD

Table A.8: Codes used to evaluate hormonal contraceptive use within the CPRD

Table A.9: Codes used to evaluate usage of hormone replacement therapy within the CPRD

Table A.10: Codes used to identify oophorectomies within the CPRD

Table A.11: Codes used to identify diagnoses of polycystic ovary syndrome within the CPRD

Chapter 1: Introduction

Breast cancer is the most commonly diagnosed form of cancer in women, worldwide (Ferlay et al., 2015). Efforts by legislative, medical, and charitable communities have aimed to curtail the disease's mortality rate through the development and implementation of novel treatment regimens and comprehensive screening programs. As a result, recent decades have seen a decrease in breast cancer-related mortality rates across the developed world (WHO International Agency for Research on Cancer, 2014). Nevertheless, age-standardized incidence rates (ASIR) for breast cancer remain significantly elevated in developed nations when compared to less developed regions of the world (Ferlay et al., 2015). While a sizeable portion of this disparity may be attributed to superior detection rates in developed nations, hormonal factors also play a role (Colditz & Bohlke, 2015). Understanding these hormonal factors is critical for identifying targets for preventative efforts.

There exists a strong interplay between a woman's longitudinal hormonal profile and their risk of developing breast cancer. This has led to debate regarding the safety of ovarian-stimulating fertility treatments. These therapies involve the administration of drugs which promote follicular growth and development within the ovaries. Drugs such as clomiphene and exogenous gonadotropins are extremely useful in the context of treating infertility as they regulate and amplify the ovulatory cycle. They are used in a variety of clinical situations, such as in treating women with ovulatory abnormalities, or in the context of assisted reproductive technologies such as in vitro fertilisation (IVF). The concern regarding these treatments stems from the effect they have on a female's hormonal profile during therapy. During ovarian stimulation, serum estrogen increases to supraphysiologic levels.

The main objective of this thesis is to evaluate the potential association between ovarian-stimulating fertility treatments and breast cancer. Evaluating this relationship is an important public health question as these treatments are becoming more common across the developed world. The association of interest will be explored in four ways. First, the hormonal etiologies of breast cancer and the mechanisms through which estrogen contributes to mammary tumorigenesis will be reviewed. Second, the framework for ovarian stimulation in the context of treating infertility will be outlined, with a focus on how these therapies alter the hormonal profiles of treated patients. Third, a systematic review of the existing literature on this topic will be presented. This review will include the results of a meta-analysis of observational studies examining this association of interest. Finally, a novel case-control study examining this relationship will be presented and evaluated in the context of the existing literature.

Chapter 2: Hormonal Etiologies of Breast Cancer, Infertility, and Ovarian-Stimulating Fertility Treatments

2.1: Breast Cancer: An Overview

Breast cancer is the most frequently diagnosed form of cancer in women worldwide, with approximately 1.7 million new cases diagnosed each year (Ferlay et al., 2015). In Canada, approximately 26,000 women are diagnosed with breast cancer annually (Canadian Cancer Society's Advisory Committee on Cancer & Statistics, 2017). In recent decades, important medical developments have led to improved breast cancer detection and treatment. For instance, more robust screening regimens have been implemented to detect breast malignancies at earlier stages of their progression (Coldman et al., 2014; Marmot et al., 2013). The discovery that breast cancer is not a homogeneous disease but should rather be subclassified into histological and molecular subtypes has spurred the development of targeted treatment strategies (Malhotra, Zhao, Band, & Band, 2010). Identifying and characterizing causative risk factors for breast cancer, such as the discovery of the BRCA1 and BRCA2 genes, or the association between hormone replacement therapy (HRT) and breast cancer, has aided in identifying high-risk populations which should be screened and treated appropriately (Balmana, Diez, Castiglione, & Group, 2009; Collaborative Group on Hormonal Factors in Breast Cancer, 1997). As a result, breast cancer's mortality rate has decreased across the developed world. For example, in Canada, the annual age-standardized mortality rate for this disease decreased from 41.7 deaths in 100,000 in 1988, to 23.2 deaths in 100,000 in 2017, a decrease of 44% (Canadian Cancer Society's Advisory Committee on Cancer & Statistics, 2017). The Canadian Cancer Society estimates that 32,000 breast cancer-related deaths in this time interval were prevented.

Unlike breast cancer-related mortality rates, the incidence of this disease in the Canadian population has remained relatively stable throughout the past three decades. The nationwide annual ASIR has fluctuated in the vicinity of 130 diagnoses per 100,000 women (Canadian Cancer Society's Advisory Committee on Cancer & Statistics, 2017). This is almost four times higher than the ASIR of developing nations, which according to the World Health Organization (WHO), is 27.3 in 100,000 (Jemal et al., 2011). This discrepancy may be explained, in part, by ASIRs in the developing world being significantly underestimated. Many cases of breast cancer remain undiagnosed as the cancer surveillance programs in these regions are simply not as robust as those in developed countries (Althuis, Dozier, Anderson, Devesa, & Brinton, 2005). Nevertheless, other factors are also involved in explaining this discrepancy. Numerous reproductive factors affect breast cancer risk. Age of menarche, age of menopause, age of first birth, and parity have all been identified as factors affecting a female's lifetime risk of developing a breast malignancy (Collaborative Group on Hormonal Factors in Breast, 2012; MacMahon et al., 1970). These factors alter a woman's longitudinal hormonal profile, which in turn affects her risk of developing breast cancer. Additionally, obesity, hormone replacement therapy (HRT), and hormonal contraceptive use also have an effect on reproductive hormone levels (Basen-Engquist & Chang, 2011; Collaborative Group on Hormonal Factors in Breast, 1996; Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Morch et al., 2017).

Women in developed regions are statistically more likely to be of lower parity, to give birth later in life, to be obese, and are more likely to use, or have used, hormonal contraceptives and HRT (Bray, McCarron, & Parkin, 2004). Furthermore, in the developed world, girls are entering puberty at much younger ages, a phenomenon that has been, in part, attributed to

environmental contaminants which act as endocrine disruptors (Karapanou & Papadimitriou, 2010). These factors all contribute to breast cancer's elevated ASIR in developed nations.

2.2 Hormonal Etiologies of Breast Cancer

The interplay between endogenous reproductive hormone levels and breast cancer has been discussed in scientific literature for almost two centuries. In 1824, the British surgeon Sir Astley Cooper published his *Practices and Principles of Surgery* in which he outlined his experiences diagnosing and treating patients with breast malignancies (Cooper & Tyrrell, 1824). Notably, he remarked "the symptoms [of breast cancer] are augmented at the approach of menstruation and decline as the period is passing (Cooper & Tyrrell, 1824)." He postulated that physiological fluctuations occurring over the menstrual period interact with the tumour, increasing its size, and aggravating symptoms. Additionally, he noted that the disease appeared to occur more frequently in women who had never given birth. At the time, the understanding of the human endocrine system was relatively rudimentary, and these observations could not be explained mechanistically, but would be evidenced in due time. The German physician Albert Schinzinger published his own findings 65 years following the work of Cooper, in 1889. He reported that cases of breast cancer in younger females were much more aggressive than those occurring in women of post-menopausal ages. Dr. Schinzinger suggested these younger women undergo oophorectomy to treat their disease (Schinziger A, 1889). This work influenced British physician G.T. Beatson, who published a case report in *The Lancet* detailing an oophorectomy performed on a woman with pre-menopausal breast cancer. The procedure resulted in a significant improvement in his patient's condition (Beatson GT, 1896). A decade later, Lett et al. published an observational study supporting this form of therapy for pre-menopausal breast malignancies. The study reported 24 of 99 pre-menopausal women with breast cancer

experienced a marked improvement in their disease status following ovarian ablation (Lett, 1905). For a period at the start of the 20th century, oophorectomy, or ovarian ablation by radiotherapy, was the standard of care for severe cases of pre-menopausal breast cancer.

At this point in time, it was understood that 'secretions' from the ovary influence a significant portion of breast cancers. The major player in these 'secretions' is, of course, estrogen, a steroid hormone characterized in 1929 by Butenandt and Doisy (Tata, 2005). Their findings formed the basis for research into breast cancer treatments which inhibit the effects of estrogen in breast tissue. For example, tamoxifen, a selective estrogen receptor modulator (SERM), acts as a competitive antagonist for estrogen receptors present in breast tissue. This drug has become the standard of care for both pre- and post-menopausal breast cancers expressing the estrogen receptor (ER). Other important forms of therapy for ER+ breast cancers are aromatase inhibitors such as letrozole and anastrozole. These drugs inhibit the functioning of the aromatase protein, the key catalyzing enzyme in the conversion of androgens to estrogens. Estrogen also plays a role in the initiation and progression phases of breast tumorigenesis. The following sections will explore the mechanisms through which this hormone modulates breast cancer risk and will provide an overview of studies which have explored this association observationally.

2.3 – The Role of Estrogen in the Development of Breast Cancer

2.3.1 – Role of Reproductive Hormones in Normal Breast Development and Functioning

The structure of the mature mammary gland is similar to that of most exocrine glands found in the human body, that is, a series of secretion-producing lobules connected by a ductal system directing secretions towards larger exocrine ducts. The mammary gland is unique in that

the most critical points in its development occur postnatally. In fact, at birth, the mammary gland is a rudimentary and dormant exocrine structure. The structure, known as the breast pad, is composed of premature lactiferous ducts and their terminal end buds (TEBs), the precursor to milk-secreting lobules (Javed & Lteif, 2013). Under normal circumstances, the breast pad will remain non-functional and indolent until puberty.

The onset of puberty commences the most critical period of breast development. With first menarche comes elevated estrogen and progesterone levels produced by the developing follicles and the corpus luteum. Ductal epithelial cells express the estrogen receptor (ER) α isoform. Upon activation, ER-α mediates downstream signalling cascades promoting cellular proliferation (Macias & Hinck, 2012). In breast tissue, ER-α signalling cascades promote lactiferous duct elongation and bifurcation into subsidiaries (Macias & Hinck, 2012). Under the influence of progesterone, the TEBs located at the distal ends of these lactiferous ducts begin their transformation into secretory acini (H. J. Lee et al., 2013). The transformation of the TEBs into secretory acini is primarily mediated by progesterone receptor (PR)-B activity through the activation of downstream Wnt and RANKL signalling pathways responsible for cellular differentiation and morphogenesis (H. J. Lee et al., 2013). Final maturation and differentiation of the breast occurs during pregnancy, as the effects of prolactin are needed for "final lactogenic differentiation (Macias & Hinck, 2012)."

2.3.2 Estrogen and Breast Cancer: Mechanism of Action

The development of breast cancer is a complex, multi-step process occurring over the span of many years. This tumorigenic process involves the accumulation of changes to cellular signalling and genetic expression which transforms normal cells into ones exhibiting abnormal

proliferation, growth regulation, and survival. This section will focus on the role of estrogen in the development of breast cancers of epithelial origin, as over 95% of breast cancers arise from the breast tissue's epithelial cells (Makki, 2015).

As mentioned in the previous section, estrogen promotes the proliferation of lobular and ductal epithelial cells within the mammary gland. Upon the binding of this steroid hormone to cytoplasmic estrogen receptors, the complex undergoes a conformational change and dimerizes. The dimers localize to the nucleus where they bind to a series of regulatory regions in DNA known as estrogen response elements. Upon binding, the complexes interact with transcription factors, coactivators, and corepressors to alter DNA transcription. Transcriptional activity of genes responsible for cell growth, proliferation, and the suppression of apoptosis is upregulated (Gompel et al., 2000; Pike, Spicer, Dahmoush, & Press, 1993). Key targets for this process are the CDK4, Cyclin D1, and c-Myc genes (Dalvai & Bystricky, 2010). Estrogens also exert effects on cellular proliferation which are not transcriptionally regulated. For example, estrogen-mediated signalling activates a group of kinases known as the mitogen-activated protein kinases (MAPs). Estrogen signalling also increases the intracellular levels of the second messenger cyclic AMP (cAMP). MAP kinase activity and increased levels of intracellular cAMP promotes cellular proliferation and the inhibition of apoptosis (Yager & Davidson, 2006).

An elevated rate of cellular proliferation is a key factor in the initiation of many solid cancers. As the rate of cell division increases, the effectiveness of DNA repair mechanisms decreases, leading to an increase in the frequency of errors during DNA replication. For example, nondisjunction events resulting in mitotic recombination occur more frequently (Yue, Yager, Wang, Jupe, & Santen, 2013). Mutations in the DNA may subsequently commence a cascade of events resulting in cellular transformation towards malignancy. The issue of genomic

instability is compounded by estrogen signalling having an inhibitory effect on apoptosis. This prevents cells which would normally undergo cell death due to an accumulation of mutations from doing so (Zhivotovsky & Kroemer, 2004).

The metabolism of estrogen is also postulated to contribute to mammary carcinogenesis. In breast tissue, estrogen is metabolized by cytochrome P-450 enzymes into catechols, namely 2-hydroxycatechol estrogen and 4-hydroxycatechol estrogen (Samavat & Kurzer, 2015; Yager & Davidson, 2006). These catechols are further metabolized into quinones, which form unstable bonds to adenine and guanine. These quinones act as DNA adducts and cause errors in DNA replication. The catechols may also enter reduction-oxidization cycling. By-products of the reduction of these catechols are reactive oxygen species, which cause significant damage to DNA (Yager & Davidson, 2006).

Estrogen signalling also influences breast cancer progression. This is due the hormone's effects on cellular proliferation, evasion of apoptosis, promotion of genome instability, and the deregulation of cell-cell interactions promoting the invasive and metastatic capabilities of pre-malignant cells. The effects of estrogen are postulated to stimulate the advancement of pre-malignant breast lesions, such as ductal hyperplasia and ductal carcinoma in situ (DCIS), into more invasive stages of the disease. For example, estrogens promote cytoskeletal remodelling and inhibit cell-to-cell interactions in pre-malignant cells. The activated estrogen receptor increases the activity of the RhoA GTPases Cdc42 and Rac. These proteins work to increase the remodelling rate of the actin cytoskeleton (Azios et al., 2007). Cytoskeletal remodelling promotes the invasive and migratory potential of transformed cells. Estrogen receptor signalling also downregulates E-cadherin expression, impairing a cell's ability to maintain its epithelial directionality (Platet, Cathiard, Gleizes, & Garcia, 2004). The hormone also acts as a promoter of

tumour angiogenesis, a key step in the pre-malignant to malignant transition. In endothelial cells, vascular endothelial factor receptors are upregulated by ER signalling, facilitating vascular angiogenesis within masses of transformed cells (Losordo & Isner, 2001).

2.3.3 Estrogen and Breast Cancer: Observational Evidence

Numerous models have been developed aiming to quantify a patient's lifetime risk of breast cancer. One such example is the Tyrer-Cuzick model (Tyrer, Duffy, & Cuzick, 2004), which is used for patient counselling and identifying candidates for more vigilant screening protocols. This model has been externally validated and shown to possess sufficient sensitivity and specificity in quantifying breast cancer risk (Amir, Freedman, Seruga, & Evans, 2010). The algorithm synthesizes a series of genetic, demographic, and clinical variables which impact breast cancer risk. Additionally, it includes a series of reproductive factors such as age of menarche, age of menopause, age of first birth, parity, HRT use, and hormonal contraceptive use. The values of these variables are integrated to determine the patient's lifetime risk of breast cancer.

Observational studies have provided much of the evidence supporting the inclusion of these reproductive factors in risk assessment models for breast cancer. The highest quality evidence comes from a series of meta-analyses conducted by the Collaborative Group on Hormonal Factors in Breast Cancer. This multi-centre collaboration aimed to compile and analyze the body of literature examining the association between hormonal factors and breast cancer. A summary of their findings is presented in Table 2.1.

Table 2.1: Hormonal Risk Factors for Breast Cancer According to the Collaborative Group on Hormonal Factors in Breast Cancer

Hormonal Factor	**Impact on Breast Cancer Risk According to the Collaborative Group on Hormonal Factors in Breast Cancer**
Age of menarche	Breast cancer risk increases by a factor of 1.050 (95% CI [1.044-1.057], $p < 0.0001$) for every year a woman is younger at first menarche (baseline mean age of 13.1 years) (Collaborative Group on Hormonal Factors in Breast, 2012).
Age of menopause	Breast cancer risk increases by a factor of 1.029 (95% CI [1.025-1.032], $p < 0.0001$) for every year a woman is older at menopause (baseline mean age of 49.3 years) (Collaborative Group on Hormonal Factors in Breast, 2012).
HRT use	Breast cancer risk in current users of HRT, or those who ceased using HRT 1 to 4 years prior, is increased by a factor of 1.023 (95% CI [1.011-1.036], $p = 0.0002$) for every year of use. The relative risk in women using HRT for more than 5 years is 1.35 (95% CI [1.21-1.49], $p < 0.0001$). The risk subsides 5 years following cessation of use regardless of the duration of use (Collaborative Group on Hormonal Factors in Breast Cancer, 1997).
Hormonal contraceptive use	Increased relative risk of breast cancer in current users (RR 1.24, 95% CI [1.15-1.33], $p < 0.00001$). Increased relative risk of breast cancer 1 to 4 years after cessation of use (RR 1.16, 95% CI [1.08-1.23], $p = 0.00001$). Increased relative risk of breast cancer 5 to 9 years after cessation of use (RR 1.07, 95% CI [1.02-1.13], $p= 0.009$). No increased relative risk of breast cancer 10 or more years following cessation of use (Collaborative Group on Hormonal Factors in Breast, 1996).

Obesity	Women with a BMI between 25.0 and 27.4 kg/m^2 had a relative risk of 1.45 (95% CI [1.08-1.95]), compared with the reference group (BMI of less than 22.5 kg/m2). Women with a BMI between 27.5 and 29.9 kg/m^2 had a RR 1.62 (95% CI [1.17-2.24]), compared with the reference group. Women with a BMI greater than 30 kg/m^2 had a RR 1.36 (95% CI [1.00-1.85]), compared with the reference group (Key et al., 2003).

The classification of elevated BMI levels as a 'hormonal factor' is in-part due to white adipose tissue's endocrine function. Adipocytes express the aromatase enzyme. Adipose tissue is therefore one of the most significant sources of peripheral estrogen production (Nelson & Bulun, 2001). As a result, higher volumes of adipose tissue in the hypodermal layer surrounding the mammary gland will lead to higher local concentrations of estrogen. Additionally, adrenal activity is often elevated in overweight and obese women, leading to higher serum concentrations of androgens, the precursor to estrogens (Pasquali, Vicennati, Cacciari, & Pagotto, 2006).

Common to the above factors is they increase the total lifetime exposure of breast tissue to estrogen. Many forms of solid cancers exhibit a log-linear increase in risk with age. As Pike et al. reported, this model does not adequately quantify a patient's risk of developing breast cancer over their lifetime. For the model to more accurately reflect breast cancer risk, it must be adjusted for total lifetime exposure to estrogen (Pike, Krailo, Henderson, Casagrande, & Hoel, 1983).

2.4: Infertility - Prevalence and Trends

The World Health Organization (WHO) defines infertility as "the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [by individuals of reproductive age] (Zegers-Hochschild et al., 2009)." A systematic analysis of 277 international surveys concluded that infertility affects over 48.5 million women worldwide (Mascarenhas, Flaxman, Boerma, Vanderpoel, & Stevens, 2012). In developed nations, infertility rates are reportedly on the rise. The 2009-2010 Canadian Community Health Survey found that approximately 500,000 couples of ages 16 to 44 experienced a failure to become pregnant within

12 months of unprotected intercourse, a prevalence of 16% among all couples (Bushnik, Cook, Yuzpe, Tough, & Collins, 2012). Comparable surveys from 1992 and 1982 reported prevalence estimates of 8.5% (Dulberg CS, 1993) and 5.4% (Balakrishnan TR, 1993), respectively. Similar trends have been documented both in the United Kingdom (UK) (Bhattacharya et al., 2009) and the United States (Martin JA, 2017).

The increasing prevalence of infertility in the developed world can be attributed to a multitude of sociological factors. Women are pursuing university-level degrees and full-time careers at unprecedented rates. In 2013, Statistics Canada reported 82.1% of women participated in the workforce, an increase from 60% in the early 1980s (Morissette, 2017). The percentage of women with a university-level degree doubled from 15% to 30% within the same period. As a result, women are increasingly delaying childbirth. Historical data from 1976 shows the average maternal age of first birth, in Canada, was approximately 24 years old. In 2011, the mean age of first childbirth had risen to 28.5 years of age (Dion P, 2014). Advanced maternal age is among the most important causes of infertility. As a woman's ovaries age, the quality of her oocytes declines appreciably, affecting fecundity (Klein & Sauer, 2001).

The prevalence of obesity in women of reproductive age is also at historically high levels. The United States National Centre for Health Statistics reported that in 2014, 34.4% of women aged 20 to 39 were clinically obese, defined as a BMI of over 30 kg/m^2 (Ogden CL, 2015). A similar nationwide survey conducted in 2002 reported 23.0% of women aged 20 to 44 had a BMI greater than 30 kg/m^2 (Vahratian, 2009). The mechanism through which obesity affects fecundity is complex and multifactorial. Observational studies have shown that obese women are at an increased risk of experiencing disruptions in their hypothalamic-pituitary-gonadal (HPG) axis. For example, a study by Jain et al. showed obese women were significantly more likely to

experience impaired luteinizing hormone (LH) pulsatility, a risk factor for ovulatory dysfunction (Jain et al., 2007). Obesity is also recognized as a major risk factor for hyperinsulinism and insulin resistance. These conditions are associated with polycystic ovary syndrome (PCOS), an important risk factor for infertility (Dag & Dilbaz, 2015).

2.5: Principles of Fertility Treatments

The overview of the principles of infertility evaluation and treatment detailed in this review are primarily based on the National Institute for Health and Care Excellence (NICE) framework (National Collaborating Centre for Women's and Children's Health (UK), 2013). NICE guidelines are funded by the UK's Department of Health and serve to standardize evidence-based care. This guideline was chosen as the information used to conduct the case-control study, presented in Chapter 4, was extracted from the Clinical Practice Research Datalink (CPRD). This database compiles medical records for patients treated by primary care practices operating in the UK.

2.5.1: Counselling and Investigation for Fertility Issues - Primary Care

Couples with difficulty conceiving and who seek counselling from a primary care physician will generally undergo a medical history review, physical examination, and assessment of lifestyle factors which may affect fecundity. Following the results of this evaluation, the physician, in conjunction with the patient(s), will orient further treatment. The physician may recommend that the couple seek care from secondary or tertiary care centres should the root cause of the infertility be readily diagnosable and require specialized forms of therapy. Immediate referral is also indicated if the female is of advanced age, as delaying specialized evaluation and treatment diminishes the probability of favourable outcomes. Additionally,

immediate referral is indicated in cases where the couple is unable to have unprotected sexual intercourse, such as in cases where one of the partners is infected with the human immunodeficiency virus.

Should immediate referral not be warranted, the couple will generally be counselled to continue unprotected intercourse while enacting lifestyle adjustments to optimize the odds of conception. Factors such as smoking, alcohol use, recreational drug use, occupational exposure to certain chemicals, and a BMI of less than 19 or greater than 30 decrease fecundity. The couple should also be encouraged to monitor the female's ovulatory cycle and perform sexual intercourse during the period surrounding ovulation to maximize the chances of conception. Should expectant management be unsuccessful, referral to a clinic specialized in treating fertility is warranted.

2.5.2: Counselling and Investigation for Fertility Issues – Specialized Care

Investigations at fertility clinics are concerned with diagnosing the root cause of the fertility issue in order to orient treatment. For females, further investigations can be classified into three main lines of testing: ovulation monitoring, ovarian reserve assessment, and imaging of the uterine cavity and fallopian tubes.

To assess for ovulatory function, the patient is asked to describe both the regularity and frequency of her menstrual cycles. Ovulation monitoring may also be considered to evaluate whether the patient is regularly ovulatory. This form of monitoring is most commonly performed by measuring serum progesterone during the mid-luteal phase of the ovulatory cycle. Generally, a serum progesterone level of less than 5ng/mL during the mid-luteal phase indicates anovulation. Females reporting irregular menstrual cycling are at a higher risk of having an

ovulatory disorder. Women who report normal menstrual cycling are most likely ovulatory, yet serum progesterone testing may still be offered to confirm ovulation.

Assessing a patient's ovarian reserve is important as a poor ovarian reserve can be indicative of either poor oocyte quality or oocyte quantity. Ovarian reserve testing is especially important in women over the age of 35 as oocyte quality deteriorates with age. There are three common forms of testing to assess the ovarian reserve: serum FSH measurement, serum Anti-Mullerian Hormone (AMH) measurement, and antral follicle count. FSH levels should be measured on day two or three of the menstrual cycle. The levels of this hormone early in the cycle correlate with the number of developing follicles within the ovaries. Should the number of developing antral follicles be low, serum estrogen levels will also be low. As a result, serum FSH levels will be elevated as estrogen-mediated inhibition of FSH release by the anterior pituitary will be decreased. AMH is a glycoprotein expressed by preantral and early antral follicles. Its levels in serum are therefore indicative of the quantity and quality of the primordial follicle pool. AMH levels may be measured at any time point of the menstrual cycle as its serum concentration remains relatively constant in this time period (La Marca et al., 2009). Low AMH levels are indicative of a poor ovarian reserve. Finally, the ovarian antral follicle count is typically quantified by transvaginal ultrasound of the ovaries. Imaging should ideally be performed in the early follicular phase of the cycle. In summary, FSH levels of more than 8.9 IU/L, AMH levels of more than 5.4 pmol/L, or an antral follicle count of less than 4 are indicative of a poor ovarian reserve. Low FSH levels may also be indicative of a poorly functioning HPG axis.

Should these two lines of non-invasive investigations indicate the infertility is due to ovulatory dysfunction, the results from ovulation tracking and ovarian reserve testing are synthesized to classify the disorder according to WHO guidelines.

WHO Group I ovulation disorders: This group includes patients suffering from hypothalamic pituitary failure, a condition known as hypogonadotropic hypogonadism (HH). The typical clinical presentation for patients in this group is amenorrhea, estrogen deficiency, and low levels of FSH due to a poorly functioning HPG axis.

WHO Group II ovulation disorders: The most common cause of Group II ovulation dysfunction is PCOS. The most common clinical features of PCOS is amenorrhea or oligomenorrhea, hyperandrogenism, and polycystic ovary morphology with an antral follicle count of greater than 12 seen on transvaginal ultrasound. Obesity is the most important risk factor for PCOS. In this condition, developing follicles will typically experience an arrest in the early antral stages and will not progress in their development.

WHO Group III ovulation disorders: This group includes patients experiencing, or having experienced, ovarian failure. Generally, these patients present with amenorrhea accompanied by high FSH levels, low AMH levels, and decreased antral follicle count.

Should no ovulatory dysfunction be found, additional testing should be performed to determine if abnormalities or conditions of the reproductive system are causing the fertility issues. Generally, these lines of testing are only performed if ovulatory factors are ruled out as they are quite invasive and costly, however, they may be performed more readily in cases where the patient presents with certain conditions or a medical history indicative of uterine or tubal dysfunction. The preeminent method of imaging the uterine anatomy and assessing the patency of the fallopian tubes is via hysterosalpingography (HSG). HSG may reveal findings such as tubal occlusions, uterine synechiae, and uterine fibroids which may be causing the fertility issues.

Endometriosis is also a common cause of infertility. This condition is characterized by the outgrowth of endometrial tissue from the uterine cavity. Most commonly, the endometrial tissue infiltrates the fallopian tubes, ovaries, and peritoneal cavity. Depending on its severity, endometriosis may impede normal reproductive functioning and affect fecundity. Approximately 30% to 50% of women suffering from endometriosis are infertile (Bulletti, Coccia, Battistoni, & Borini, 2010).

Assessment of male-factor infertility includes similar lines of testing to that of female-factor infertility. Physical examination, endocrine testing, imaging of the male reproductive system, and semen analyses are common investigative tools. Male-factor infertility generally manifests itself in the form of low semen volume, low sperm concentration within semen, poor sperm motility, or abnormal sperm morphology.

Finally, it should be noted that a sizeable portion of infertility cases, 15% to 30%, have an unidentifiable root cause following the normal lines of investigation (Quaas & Dokras, 2008).

2.5.3: Treatment of Infertility – Specialized Care

Treatment of infertility is oriented according to the underlying cause. In certain situations, expectant management may be attempted before more invasive or costly forms of therapy are considered. For example, PCOS in obese women may, in part, be caused by insulin resistance. In such cases, the patient will be advised to lead a healthier lifestyle which may lead to a resolution of the condition. For women with WHO Type I ovulatory disorders, the decreased activity of the pituitary gland may be caused by an extremely low BMI or an excessive amount of strenuous exercise. A summary of the treatment protocols according to underlying cause is outlined in Table 2..

Table 2.2: Treatment of Infertility According to Underlying Cause

Cause of Infertility	Treatment
Ovulation Disorders	
WHO Group I ovulation disorders	Pituitary insufficiency should be addressed through gonadotropin replacement therapies to mimic normal pituitary function. The gonadotropin formulations used should have both FSH and LH activity. Human chorionic gonadotropin (hCG) may be administered to mimic the LH surge and trigger ovulation. IVF may be offered should gonadotropin-based therapies prove unsuccessful.
WHO Group II ovulation disorders	Should expectant management in women with Group II ovulation disorders prove unsuccessful or not be indicated, ovarian stimulation with clomiphene is the first line of therapy. The drug is administered on day 2 of the cycle for a period of 5 days, with a starting dose of 50 mg per day. This dose may be increased depending on the patient's response. Clomiphene may also be supplemented with metformin. In certain cases, clomiphene is unsuccessful in normalizing the ovulatory cycle, even at higher doses. Should this occur, gonadotropin-based stimulation protocols may be attempted. IVF may be offered if these efforts fail, or if IVF is indicated.
WHO Group III ovulation disorders	No forms of ovarian stimulation should be attempted. A common course of treatment is IVF with donor oocytes.

Tubal-factor	Management of tubal-factor infertility is dependent on the severity, location and cause of the tubal abnormality. Tubal surgery may be opted for in cases where surgery offers a good prognosis. For example, in cases where the fallopian tubes are obstructed proximally, salpingography with tubal catheterization may be indicated. Salpingectomy may be offered should the tubal occlusion be the result of a hydrosalpinx. Should the tubal disease be severe, IVF may be offered. Generally, treatment is oriented according to prognosis, cost, and patient input.
Uterine Abnormality	Surgical correction of the abnormality may be necessary should the uterine abnormality be directly impeding embryo implantation within the endometrium. This may be true in cases of uterine fibroids, adhesions, or endometrial polyps. For example, women presenting with amenorrhea caused by intrauterine adhesions may be offered hysteroscopic adhesiolysis to restore menstruation. In cases of severe malformations, surgical correction may be improbable, and a gestational carrier will be needed.
Endometriosis	Treatment for women suffering from endometriosis depends on the stage of the disease. Staging may be performed in accordance with American Society of Reproductive Medicine guidelines (American Society for Reproductive Medicine, 1997). In mild cases, conception through sexual intercourse or intrauterine insemination (IUI) may be still be possible. In severe cases, surgery may be considered to resect the outgrowths of endometrial tissue. IVF may also be considered.

Male-factor	Treatment for male-factor infertility is outside of the scope of this review, however, in cases of severe deficits in semen quality and quantity, intracytoplasmic sperm injection (ICSI) may be necessary.
Unexplained	Ovarian stimulation treatments should not be offered in cases of unexplained infertility. Instead, the couple should undergo expectant management while continuing attempts at conception for a period of 12 to 24 months. Should the issues persist, IVF may be considered.

2.6: Overview of Ovarian-Stimulating Fertility Treatments

The purpose of this section is to explore ovarian-stimulating fertility treatments in greater detail. This includes clomiphene therapy, gonadotropin therapy, IVF, and ICSI. The stimulation protocols for IVF are identical to those of ICSI. Therefore, any mention of IVF in the following section should be treated as a proxy for ICSI.

2.6.1: Clomiphene

Clomiphene is classified as a selective estrogen receptor modulator (SERM). SERMs are structurally similar to estrogens, owing to their stilbene cores, allowing them to cross cell membranes and bind to cytoplasmic estrogen receptors. SERMs may act as either estrogen receptor agonists or antagonists depending on the target tissue. Site-specific activity is regulated by the interaction between the SERMs and coactivators or corepressors involved in the transcription of estrogen-response genes as these interactions are cell-type specific (Feng & O'Malley, 2014).

Clomiphene's utility in ovarian stimulation is due to its mechanism of action at the level of the hypothalamus, anterior pituitary, and the ovaries. In the hypothalamus, clomiphene binds to estrogen receptors and acts as an antagonist, thereby inhibiting estrogen receptor signalling. This decreases estrogen-mediated inhibition of gonadotropin-releasing hormone (GnRH) release by the hypothalamus. As a result, GnRH is released from the hypothalamus into the anterior pituitary in a pulsatile fashion (Adashi, 1984). Within the anterior pituitary and in the ovaries, clomiphene acts as an ER agonist. In the anterior pituitary, clomiphene sensitizes gonadotropin-secreting cells to GnRH, mimicking estrogen-mediated sensitization of the anterior pituitary to the effects of GnRH (Adashi, Hsueh, Bambino, & Yen, 1981). In the ovaries, clomiphene has

been shown to sensitize granulosa cells within the developing follicles to the effects of FSH, thereby promoting follicular growth and aromatase activity within these cells (Schwartz, Brezinski, & Laufer, 1993). In summary, clomiphene is directly involved in processes taking place in the hypothalamus, anterior pituitary, and ovaries to initiate follicular recruitment, growth, and development.

Clomiphene also affects other estrogen-sensitive parts of the body such as the vagina, cervix, and uterus. In these tissues, clomiphene acts as an ER antagonist. As such, these organs respond to clomiphene treatment in a manner similar to their response during periods of low estrogen levels. For example, in clomiphene-initiated ovulatory cycles, the uterine volume does not increase as it would during unstimulated cycles, and the typical endometrial thickening seen during normal cycles is reduced in magnitude (Eden et al., 1989). Furthermore, a meta-analysis has shown that when clomiphene doses exceed 100 mg per day, the production of cervical mucus is significantly reduced (Roumen, 1997).

Generally, clomiphene administration commences on day 2 of the menstrual cycle and is continued for a period of 5 days. Response to treatment may be monitored by transvaginal ultrasound of the ovaries. In cases where the timing of ovulation is important, such as cases where intrauterine insemination (IUI) is being used, ovulation may be triggered with the use of human chorionic gonadotropin (hCG).

2.6.2: Gonadotropins

Gonadotropin-based therapies may either be used as standalone treatments or as a part of procedures such as IVF. For the purposes of this review, the use of gonadotropins as standalone treatments will be discussed first, followed by a discussion about their role in IVF. As standalone

treatments, the goal is similar to that of clomiphene-based treatments, that is, the normalization of the ovulatory cycle. Unlike clomiphene, which stimulates the release of endogenous gonadotropins from the anterior pituitary, this form of treatment involves the administration of exogenous gonadotropins to directly raise their serum levels.

Gonadotropin preparations may either be urinary or recombinant. The urinary version is commonly referred to as human menopausal gonadotropin (hMG). hMG is produced by purifying FSH and LH from the urine of postmenopausal women, as postmenopausal women typically have high circulating levels of these hormones. Certain urinary preparations contain both FSH and LH (Pergonal, Humegon), while other versions have eliminated most of the LH from the preparation (Bravelle, Metrodin). Most recombinant preparations contain either solely FSH (Gonal-F, Follistim), or solely LH (Luveris). In most cases, FSH-only preparations are adequate for restoring the normal ovulatory cycle. Randomized control trials have demonstrated that in normo-gonadotropic women, FSH-only preparations had similar effectiveness compared to preparations containing both FSH and LH (Weiss et al., 2015). However, in certain cases, preparations containing both FSH and LH must be used. This is essential for stimulating follicular development in patients with impaired LH release from the anterior pituitary, such as those who suffer from HH (Gardner, Weissman, Howles, & Shoham, 2018).

As a standalone treatment, gonadotropin administration typically commences on day 2 or 3 of the menstrual cycle. Gonadotropin dosages are determined on a case by case basis. When they are used as a standalone treatment, where the goal of treatment is mono-follicular development, the therapeutic window may be relatively small. Low dosages may fail to effectively stimulate follicular development. High dosages may cause multi-follicular development, and subsequently, multiple pregnancy. High dosages also increase the risk of

ovarian hyperstimulation syndrome (OHSS) (Fiedler & Ezcurra, 2012). Regardless of the initial dosage, follicular development should be closely monitored through transvaginal ultrasound to assess the patient's response to treatment. Dosages may then be adjusted depending on the results of monitoring.

Gonadotropin-based stimulation protocols are also used in treatments such as IVF. IVF is typically reserved for cases where previous attempts at ovarian stimulation using clomiphene or gonadotropins have proved unsuccessful, or when IVF is indicated. Cases where IVF is indicated include, but are not limited to, tubal pathologies which cannot be resolved routinely through surgery, unexplained infertility lasting longer than 2 years, endometriosis, and severe cases of male-factor infertility (Gardner et al., 2018).

Ovarian stimulation for IVF differs from the forms of ovarian stimulation discussed previously as it aims to elicit multi-follicular development. The clomiphene-based and gonadotropin-based protocols discussed previously aimed to elicit mono-follicular development to prevent multiple pregnancy after sexual intercourse or IUI. Multi-follicular development allows for the retrieval of multiple follicles which can then be inseminated and incubated *in vitro.* The embryos are incubated until they are ready for transfer into the woman's uterus, which may be anywhere from the 2-cell embryonic stage to the expanded blastocyst stage. Embryos are evaluated and graded in accordance with multiple criteria which have been identified as having an impact on outcome (Cutting et al., 2008). A selected number of embryos will then be transferred. This number is determined on a case-by-case basis, by seeking to balance successful pregnancy with the risk of multiple pregnancy (Joint SOGC-CFAS, 2008).

Ovarian stimulation protocols for IVF typically require the suppression of the normal ovulatory cycle. Without this suppression, cycle cancellation can occur in up to 20% of cases due

to a premature endogenous LH surge prior to follicle retrieval (Gardner et al., 2018). Two main protocols are used to achieve this suppression and stimulate follicular development for IVF. The first involves the use of a GnRH agonist, otherwise known as the long protocol. The second involves the use of a GnRH antagonist, otherwise known as the short protocol.

Long protocol for IVF: Intervention begins one week prior to the desired start of the ovulatory cycle, on day 21 of the preceding ovulatory cycle. GnRH is administered daily for 14 days. In response to GnRH administration, FSH and LH are released from the anterior pituitary. Prolonged elevated levels of GnRH cause a downregulation in the number of GnRH receptors expressed in the anterior pituitary, thereby desensitizing the gland to endogenous GnRH. Additionally, estrogen and progesterone are produced by the ovaries in response to the gonadotropin surge at the beginning of GnRH administration. These hormones exert a negative feedback at the level of the hypothalamus, thereby inhibiting endogenous GnRH production. On day 2 of the current ovulatory cycle, exogenous gonadotropins are administered to stimulate follicular growth and development.

Short protocol for IVF: The principle of the short protocol is similar to that of the long protocol and only differs in the manner in which pituitary suppression is achieved. Here, a GnRH antagonist is administered in conjunction with the commencement of gonadotropin administration. Endogenous GnRH signaling at the level of the anterior pituitary is suppressed, inhibiting the endogenous ovulatory cycle.

To stimulate multi-follicular development, elevated levels of FSH are needed to override normal processes of follicular selection which would result in the development of a singular dominant follicle (Gardner et al., 2018). Dosages are determined on a case-by-case basis and can be adjusted based on the follicular response to stimulation. Once the follicles are sufficiently mature, hCG is used to stimulate final oocyte maturation and its release from the ovary.

2.6.3: Hormone Levels during Ovarian Stimulation

At the beginning of the ovulatory cycle, serum estrogen and progesterone levels decline as the corpus luteum produced by the previous ovulatory cycle atrophies. This leads to pulsatile GnRH release from the hypothalamus. GnRH stimulates the release of FSH from the anterior pituitary. FSH is responsible for the recruitment and development of a cohort of antral follicles within the ovaries. This is mediated by FSH receptors expressed in the granulosa cells of these antral follicles. In response to FSH, granulosa cells start to synthesize estrogen. Rising estrogen levels exert a negative feedback on FSH release from the anterior pituitary. As the level of circulating FSH declines, follicular selection occurs. One follicle from the cohort, termed the 'dominant follicle', will continue its growth and development in an FSH-independent manner until its ovulation. The rest of the cohort, under normal circumstances, will atrophy.

The cohort of developing follicles are hubs for estrogen production as their granulosa cells express the aromatase enzyme. The 'dominant follicle' also produces a substantial amount of estrogen throughout its growth. Consequentially, serum estrogen levels reach their peak in the days preceding ovulation. In the normal ovulatory cycle, the serum concentration of this estrogen peak ranges between 300 to 600 pg/mL (Reed & Carr, 2000).

During clomiphene-stimulated cycles, serum estrogen levels are elevated. A study by Reed et al. reported the mean peak serum estrogen level in women receiving clomiphene-based ovarian-stimulation was 1228 pg/mL, approximately a five-fold increase from levels during a normal ovulatory cycle (Reed et al., 2015). Unfortunately, this study did not report the precise dosage of clomiphene used. Another study corroborated these findings, reporting a mean peak estrogen level of 1150 pg/mL in women receiving 150 mg of clomiphene per day for a 5-day period at the start of the cycle (Hunlich, Trotnow, Mulz, & Kniewald, 1984).

The increased levels of estrogen seen in clomiphene-stimulated cycles can be attributed to two main factors. Firstly, aromatase activity within granulosa cells of the developing follicle is increased. Aromatase synthesis and activity within granulosa cells is directly dependent on FSH-mediated signaling (Parakh et al., 2006; Steinkampf, Mendelson, & Simpson, 1987). Clomiphene indirectly promotes the release of FSH from the anterior pituitary due to clomiphene-mediated sensitization of gonadotropin-releasing cells in the anterior pituitary to the effects of GnRH (Adashi et al., 1981). Additionally, clomiphene sensitizes granulosa cells to the effects of FSH. This is evidenced by a study by Zhuang et al. where granulosa cells were cultured, *in vitro*, with FSH. Granulosa cells cultured with both clomiphene and FSH produced higher amounts of estrogen than cells cultured solely with FSH (Zhuang, Adashi, & Hsuch, 1982). Secondly, clomiphene-stimulated cycles are more likely to result in multi-follicular development, in part due to the augmented levels of FSH seen during these cycles (Coughlan, Fitzgerald, Milne, & Wingfield, 2010). Multi-follicular development occurs in approximately 17% of clomiphene-stimulated cycles (Amer, Smith, Mahran, Fox, & Fakis, 2017). As stated previously, follicular selection occurs during the time period of decreasing FSH levels in the follicular phase of the menstrual cycle. The timing of this decline is crucial. Should FSH concentrations remain elevated for a longer than normal period of time, multi-follicular development may occur, resulting in the development of more than one ‘dominant follicle’ (Baerwald, Adams, & Pierson, 2012).

Gonadotropin-based stimulation protocols also raise serum estrogen to supraphysiologic levels. In treatments such as IVF, exogenous FSH is administered at the beginning of the follicular phase to stimulate antral follicle growth and development. FSH administration continues throughout the period of follicular selection to promote the development of multiple

pre-ovulatory follicles (Gardner et al., 2018). These follicles may then be collected for selection and insemination. Ou et al. studied over 3500 IVF and ICSI cycles that used either the long or short protocol for ovarian stimulation (Ou, Xing, Li, Xu, & Zhou, 2015). They concluded that in women undergoing the long protocol, 8 to 13 follicles were retrieved per stimulated cycle, with pre-ovulatory estrogen levels ranging between 1935 and 2821 pg/mL. For women undergoing the short protocol, 5 to 10 follicles were collected, and pre-ovulatory estrogen levels ranged between 1782-2791 pg/mL. The estrogen levels reported in this study are 5 to 15 times higher than the pre-ovulatory estrogen levels seen in women ovulating naturally. Another study by Joo et al. did not stratify their analysis on the basis of protocol type but reported an average of 10.1 oocytes were collected, per cycle, in women undergoing IVF. The average pre-ovulatory serum estrogen concentration was 3700 pg/mL (Joo et al., 2010).

2.7: Objectives and Rationale

The etiology of a significant portion of breast cancers has a hormonal component. Ovarian-stimulating fertility treatments raise serum estrogen significantly in comparison to the natural ovulatory cycle, raising questions regarding the impact of these treatments on breast cancer development. This issue has become increasingly relevant as the prevalence of infertility, and the prevalence of ovarian-stimulating fertility treatments, increases across the developed world. The total number of IVF cycles performed in fertility clinics annually has been on the rise in Canada, the UK, and the United States since the start of the 1990s. A Canadian nationwide database collecting information on the use of assisted reproductive technologies (ART), named the Canadian ART Register, reported that in the year 2000, 19 fertility clinics operating across the country performed a total of 4,685 IVF cycles (Gunby, Daya, Fertility, & Andrology, 2005).

By 2016, the number of cycles had tripled to 15,344, and the number of second and tertiary centres providing specialized fertility treatments had nearly doubled to 34 (Canadian Assisted Reproductive Technologies Registry Plus, 2017). In the UK the number of IVF cycles performed nationwide rose from approximately 7,000 cycles in 1991, to over 60,000 cycles in 2014 (Human Fertilisation and Embryology Authority, 2016). In the United States, IVF-related information has been compiled by the Center for Disease Control since 2005, when an estimated 130,000 cycles of IVF were performed nationwide. In 2014, that number had increased to approximately 170,000 (Centers for Disease Control and Prevention: American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2016).

A substantial number of Canadian provinces have approved, or are deliberating, legislation to include IVF in provincial healthcare coverage, which further emphasizes the need to understand the risks associated with these therapies. Québec was the first province to provide public funding of IVF as of August 2010 (Tulandi, King, & Zelkowitz, 2013). Ontario followed suit by including IVF in its provincial health care plan in late 2015 (Gotz & Jones, 2017). Manitoba and New Brunswick also provide financial assistance in the form of tax credits (Motluk, 2016). Recently, lobbying groups have formed in Saskatchewan, British Columbia, Alberta, and Nova Scotia to support the addition of IVF to the provincial health care coverage. The trend towards attributing public funds to cover IVF, in conjunction with rising infertility rates in Canada, will undoubtedly increase the use of IVF in the country, underscoring the need to understand the risks involved with this procedure.

The relationship between ovarian-stimulating fertility treatments and breast cancer will be further evaluated in three ways:

1) A systematic review of the current literature. Observational studies examining the association between ovarian stimulation and breast cancer will be reviewed and their results will be compiled into a meta-analysis. The results and a discussion of this analysis will be presented in Chapter 3.
2) A case-control study using patient records compiled in the UK's Clinical Practice Research Datalink (CPRD). The results from this case-control study will be presented in Chapter 4.
3) A qualitative evaluation of these two studies in the context of the literature, in addition to further research perspectives for future studies analyzing this relationship, presented in Chapter 5.

Chapter 3: Systematic Review and Meta-Analysis

The following chapter includes a manuscript detailing the methods and results from a systematic review and meta-analysis of the published literature exploring the association between ovarian-stimulating fertility treatments and breast cancer. To limit redundancy with the previous chapters, the introduction and a section on the rationale were removed as these sections contained similar information to that presented in Chapters 2 and 3.

Ovarian-Stimulating Fertility Treatments and the Risk of Breast Cancer: A Systematic Review and Meta-Analysis

Adriano Petrangelo, BSc[1]

Haim A. Abenhaim, MD, MPH [1, 2]

Montréal, Québec, Canada

[1] Department of Obstetrics and Gynecology, Jewish General Hospital, McGill University, Montréal, Québec, Canada

[2] Centre for Clinical Epidemiology and Community Studies, Jewish General Hospital, Montréal, Québec, Canada

No funding was received for this study.

Authors have no conflicts of interest to report.

Corresponding Author:

Haim A. Abenhaim, MD, MPH, FRCSC

Jewish General Hospital, Obstetrics & Gynecology, McGill University

Pavilion H, Room 325

5790 Cote-Des-Neiges Road,

Montréal, Québec, H3S 1Y9

Tel: 514-340-8222 x5488

Fax: 514-340-7941

Email: haim.abenhaim@gmail.com

Abstract

Background and Importance: The association between reproductive hormone levels and breast cancer has been an area of interest for quite some time lending credence to the hypothesis that fertility drugs, which stimulate follicular growth and development, may also modify breast cancer risk. During treatment, the serum estrogen levels of patients rise to supraphysiologic levels. Treatments such as IVF, which primarily makes use of exogenous gonadotropins, and clomiphene have been the subject of numerous observational studies examining the association between these treatments and breast cancer. We sought to review the literature and aggregate eligible observational studies into a meta-analysis to determine if a quantitative synthesis of these studies supported any potential association.

Methods: A literature search of the EMBED and MEDLINE databases was performed to identify both cohort and case-control studies examining the association between ovarian-stimulating fertility treatments and breast cancer. A random effects meta-analysis was performed using the DerSimonian and Laird pooling model to calculate a pooled measure of effect. Studies were weighted according to the inverse-variance weighting method.

Results: Our analysis included 17 cohort studies and 4 case-control studies. No significant association was found between breast cancer and clomiphene use, gonadotropin use, or a combination of the two. A subgroup analysis stratified subjects on the duration of follow-up time. Ovarian-stimulating fertility treatments were not associated with and increased risk breast cancer in all strata of subject follow-up time.

3.1: Materials and methods

3.1.1: Criteria of studies selected

All observational studies examining the association between ovarian-stimulating fertility treatments and breast cancer were considered for potential inclusion in this review. Only studies published after January 1st, 1995 were considered for inclusion to ensure studies possessed sufficient follow-up time of their study population. IVF became noticeably popular in the mid-to-late 1980s, shortly after the first IVF treatment was performed in 1978. This cut-off date ensured studies possessed the possibility of having a follow-up time of at least 10 years. All qualitative reviews, meta-analyses, case series, and case reports were excluded. Abstracts for which a full text was unavailable were excluded. A study must have included a minimum of 10 cases of breast cancer in the exposed cohort to ensure their statistical analysis had sufficient power. Only studies which provided a clear representation of their source population were considered for inclusion. Studies must have had a unique study population which had not been used in any other included study. Therefore, for studies which published two or more analyses using the same, or overlapping, study populations, the latest study was included, and the previous analyses were excluded. There were no criteria for control subjects, however, the study must have clearly stated the population from which controls were selected. Studies must have been available in the English language. Finally, a study must have clearly stated its exposure(s) of interest and provided sufficient details regarding the types of drugs included in their exposure definition.

3.1.2 Exposures assessed

Ovarian-stimulating treatments were broken down into three main categories: clomiphene use, gonadotropin use, and clomiphene used in conjunction with gonadotropins. Concerning the gonadotropins, measures of effect evaluating the relationship between human chorionic gonadotropin (hCG) and breast cancer were excluded. In fertility treatments, hCG is used to mimic the LH surge which occurs in the middle of the normal ovulatory cycle to stimulate final oocyte maturation and trigger ovulation. Therefore, hCG itself does not directly increase the levels of circulating estrogen. Studies such as Bernstein et al.'s, which had hCG as its exposure of interest, were excluded (Bernstein, Hanisch, Sullivan-Halley, & Ross, 1995). Another example was the study by Burkman et al., which reported a measure of effect for both hMG and hCG (Burkman et al., 2003). In this case, the measure of effect for hCG was excluded, whereas hMG's measure of effect was included. Studies with IVF as an exposure of interest were grouped into the gonadotropin group. van den Belt-Dusebout et al.'s study reports that some subjects who underwent IVF had clomiphene included in their stimulation protocol, however, the study reported that this was the case for only a small percentage (<10%) of the exposed population (van den Belt-Dusebout et al., 2016). Therefore, it was treated as a gonadotropin-only study.

3.1.3: Objectives

The primary objective of this study was to conduct a systematic review of the literature and identify all studies examining the association between ovarian-stimulating drugs and the risk of breast cancer. There is considerable interest in this topic due to the effect these treatments have on serum estrogen levels. With the rising prevalence of infertility in the developed world, these fertility treatments have become more common. A multitude of studies have been

published examining this relationship, yet there is considerable inter-study heterogeneity in multiple aspects such as design, exposure definition, and source population which makes interpretation difficult. This analysis aims to synthesize these findings.

A secondary objective of our study was to evaluate whether subject follow-up time affected the relationship of interest. Breast cancer is a disease with a substantially long latency period. The period from tumor initiation due to a carcinogenic insult to its progression into a clinically detectable mass is substantial, sometimes spanning over a decade (Olsson, Baldetorp, Ferno, & Perfekt, 2003; P. D. Terry, Miller, & Rohan, 2002; Wanebo, Johnson, Sato, & Thorslund, 1968). To appropriately observe whether ovarian-stimulating fertility treatments may be associated with breast cancer initiation or progression in its early stages, extended periods of follow-up may be required.

3.1.4: Search and selection methods

We carried out a comprehensive search of studies published between January 1st, 1995 and January 1st, 2017. The algorithm of terms used to conduct the literature search can be found in the appendix. This algorithm was used to search both the EMBASE and MEDLINE databases. Additionally, while meta-analyses were excluded from our study, the reference lists from two such studies (Sergentanis et al., 2014; Zreik et al., 2010) were reviewed to identify any studies which were unaccounted for in the initial search. All titles, abstracts, and full texts were extracted into the reference manager Endnote X7. Duplicates were removed. Study titles and abstracts were screened for relevancy to the primary objective. The full texts of studies selected for further review were read and evaluated in accordance with the inclusion and exclusion criteria.

3.1.5: Data extraction

The following variables were extracted from the included studies: the nation in which the study was conducted, study design, study population size, enrollment period, type(s) of exposures assessed, type of control group used, mean follow-up time for the study population, covariates adjusted for in the analysis, and the reported adjusted measure(s) of effect along with their corresponding 95% confidence interval(s). For cohort studies reporting multiple distinct types of effect measures, for example, providing both a relative risk, hazard ratio, or odds ratio as well as a standardized incidence ratio (SIR), the unstandardized effect measure was preferred.

3.1.6 Statistical Analysis

All odds ratios (ORs), standard incidence ratios (SIRs), and hazard ratios (HRs) were considered to be estimates of the risk ratio (RR). Due to the relative rarity of breast cancer in the general population, ORs, SIRs and HRs are reliable estimators of the RR (McNutt, Wu, Xue, & Hafner, 2003; Spruance, Reid, Grace, & Samore, 2004). This allowed for the pooling of a single type of effect measure.

Following stratification on the basis of exposure type and study design, risk ratios and their 95% confidence intervals were plotted with the use of forest plots. Considerable heterogeneity existed between studies in terms of study period, type of control group used, and covariates adjusted for. Therefore, the use of a random effects meta-analysis was deemed to be more appropriate as it was not possible to assume all included studies were functionally identical (Borenstein, 2009). Studies were assigned weights using the inverse-variance weighting method (C. H. Lee, Cook, Lee, & Han, 2016). In this method, the weight designated to each study is inversely proportional to the study's standard error. As standard error is inversely related to the

size of a study's population, studies including a larger number of subjects were assigned greater weights. Pooling was performed in accordance with DerSimonian and Laird method (DerSimonian & Laird, 1986). The z-statistic for overall effect was employed to test whether the overall measure of effect indicated a significant association. Variation across studies was assessed with the use of the I^2 statistic. This statistic measures the proportion of between-study variation attributable to inter-study heterogeneity (Higgins & Thompson, 2002). I^2 values were interpreted as follows: values below 25% indicated minimal heterogeneity, values between 25% and 75% indicated moderate heterogeneity, and values above 75% indicated high heterogeneity (Huedo-Medina, Sanchez-Meca, Marin-Martinez, & Botella, 2006).

A secondary analysis was performed to examine the effect of follow-up time on the relationship of interest. Studies which provided a stratified analysis based upon subject follow-up time were included in this portion of the analysis. Measures of effect were grouped into those for subjects followed less than 10 years, between 10 and 19 years, and more than 20 years. We chose to ignore exposure type in this secondary analysis as there were too few studies which stratified on follow-up time for this to be feasible. All analyses were performed with the usage of the Review Manager software program (Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre).

3.2: Results

The search of both the MEDLINE and EMBASE databases resulted in 1,224 unique studies considered for inclusion. The titles and abstracts of all 1,224 studies were screened for potential relevance to our study's objective. 1,084 studies were excluded following title and abstract review. A common reason for exclusion was that the study dealt with the safety of

fertility treatments in women undergoing treatment for existing breast cancer or in women with a history of breast cancer. Full-text analysis of the remaining 140 studies was necessary to evaluate their compliance with the inclusion and exclusion criteria. Of these, 70 studies were removed as they were deemed to be irrelevant to our study's objective, and 32 were removed as they were qualitative reviews, meta-analyses, case reports, case series, and *in vitro* studies. Five studies were excluded due to issues with their exposure type, definition, or assessment. Bernstein et al. was excluded as its sole exposure of interest was hCG (Bernstein et al., 1995). Petro-Nustas et al. was excluded as it classified its exposure of interest as 'fertility drugs' and did not elaborate on what types of medications was included in the exposure definition (Petro-Nustas, Norton, & al-Masarweh, 2002). Similarly, Ricci et al. and Braga et al. identified the exposed population as 'women having undergone fertility drug use' and did not elaborate on the type of fertility drugs included in the exposure definition (Braga et al., 1996; Ricci, Parazzini, Negri, Marsico, & La Vecchia, 1999). Doyle et al. was excluded as it defined its exposed population as women being treated for infertility at a fertility clinic (Doyle, Maconochie, Beral, Swerdlow, & Tan, 2002). The authors did not stratify their analysis on the type of fertility treatment used nor could they confirm all subjects included in the exposed population underwent some form of ovarian-stimulating fertility treatment. One study, Kotsopoulos et al., was excluded due to its cohort criteria. This study restricted the cohort to women with a BRCA 1 or BRCA 2 mutation (Kotsopoulos et al., 2008). Finally, 4 studies were excluded due to issues regarding their outcome of interest. Katz et al. was excluded as it was concerned with covariates which may influence the relationship between IVF and breast cancer (Katz et al., 2008). Siegelmann-Danieli et al. was excluded as it was concerned with the prognostic features of breast cancers diagnosed following fertility treatments (Siegelmann-Danieli et al., 2003). Similarly, Turkoz et al. was

excluded as it was concerned with the association between IVF and molecular subtypes of breast cancer (Turkoz et al., 2013). Finally, Kessous et al. was excluded as it simply stated the results of their study qualitatively but did not provide any measures of effect for the relationship between IVF and breast cancer (Kessous, Davidson, Meirovitz, Sergienko, & Sheiner, 2016).

3.2.1 Studies Identified

A total of 28 observational studies were identified following inspection of their full-texts. Seven of these studies had study populations overlapping with the study populations of other identified studies (Brinton et al., 2004; Kallen, Finnstrom, Nygren, Otterblad Olausson, & Wennerholm, 2005; Kristiansson, Bjor, & Wramsby, 2007; Lerner-Geva et al., 2003; Modan et al., 1998; Potashnik et al., 1999; Venn et al., 1995). In these cases, the latest study, chronologically, was included and the earlier one was excluded. Seventeen cohort studies (Brinton et al., 2014; Brinton et al., 2013; Calderon-Margalit et al., 2009; Dor et al., 2002; dos Santos Silva et al., 2009; Gauthier, Paoletti, Clavel-Chapelon, & group, 2004; Kallen et al., 2011; Lerner-Geva et al., 2006; Lerner-Geva et al., 2012; Orgeas et al., 2009; Pappo et al., 2008; Reigstad et al., 2015; Rossing, Daling, Weiss, Moore, & Self, 1996; Stewart et al., 2012; K. L. Terry, Willett, Rich-Edwards, & Michels, 2006; van den Belt-Dusebout et al., 2016; Venn, Watson, Bruinsma, Giles, & Healy, 1999) and four case-control studies (Burkman et al., 2003; Fei, Deroo, Sandler, & Weinberg, 2012; Jensen, Sharif, Svare, Frederiksen, & Kjaer, 2007; Yli-Kuha, Gissler, Klemetti, Luoto, & Hemminki, 2012) were included in the analysis. These studies were conducted in a diverse array of nations, including the Netherlands, Norway, the United States, Israel, Finland, Sweden, Australia, the United Kingdom, France, and Denmark.

3.2.2 Breast Cancer Risk Associated with Clomiphene Use

Ten cohort studies (Brinton et al., 2014; Brinton et al., 2013; Calderon-Margalit et al., 2009; dos Santos Silva et al., 2009; Gauthier et al., 2004; Lerner-Geva et al., 2006; Lerner-Geva et al., 2012; Orgeas et al., 2009; Rossing et al., 1996; K. L. Terry et al., 2006) and three case-control studies (Burkman et al., 2003; Fei et al., 2012; Jensen et al., 2007) examined the association between exposure to clomiphene and breast cancer. A total of 1,115 cases of breast cancer across both types of study design were exposed to clomiphene. Although it examined the relationship between clomiphene and breast cancer, Venn et al. was excluded from the analysis as it reported fewer than 10 cases of breast cancer in the exposed cohort (Venn et al., 1999). Forest plots for the cohort and case-control studies are found in Figures 3.2 and 3.3, respectively. Only one cohort study, Lerner-Geva et al., reported a statistically significant association for this relationship (Lerner-Geva et al., 2006). No case-control study reported a significant association.

The random-effects pooled RR for the cohort studies was 1.06, 95% CI [0.93-1.20], z-test for overall effect was non-significant ($p = 0.38$). The pooled RR for the case-control studies was 0.97, 95% CI [0.82-1.15], z-test for overall effect also non-significant ($p = 0.73$). The I^2 value for heterogeneity between the cohort studies was 38%, indicating moderate heterogeneity, whereas the I^2 value for the case-control studies indicated low heterogeneity, $I^2 = 11\%$.

3.2.3 Breast Cancer Risk Associated with Gonadotropin Use

Nine cohort studies (Brinton et al., 2014; Brinton et al., 2013; Dor et al., 2002; Gauthier et al., 2004; Kallen et al., 2011; Pappo et al., 2008; Reigstad et al., 2015; Stewart et al., 2012; Venn et al., 1999) and four case-control studies (Burkman et al., 2003; Fei et al., 2012; Jensen et al., 2007; Yli-Kuha et al., 2012) examined the association between gonadotropin use and breast

cancer. One cohort study reported this relationship as significant (Reigstad et al., 2015). No case-control study reported a significant association. Forest plots for these studies, stratified by study design, are displayed in Figures 3.4 and 3.5.

The pooled risk ratio for the cohort studies was 1.03, 95% CI [0.94-1.14], z-test was non-significant (p = 0.52). The pooled risk ratio for the case-control studies was 1.18, 95% CI [0.94-1.49], z-test for overall effect was non-significant (p = 0.16). The I^2 value for heterogeneity between the cohort studies was 52%, indicating moderate heterogeneity, whereas the I^2 value for the case-control group showed minimal heterogeneity, $I^2 = 0\%$.

3.2.4: Breast Cancer Risk Associated with Clomiphene and Gonadotropins

Six cohort studies examined the association between breast cancer and the usage of both clomiphene and gonadotropins (Brinton et al., 2014; Dor et al., 2002; dos Santos Silva et al., 2009; Lerner-Geva et al., 2006; Lerner-Geva et al., 2012; Orgeas et al., 2009). A forest plot of the results from these studies is found in Figure 3.6. None of the cohort studies reported a statistically significant association. Only one case-control study studied this relationship and reported an OR of 0.73, 95% CI [0.43-1.24] (Fei et al., 2012). Therefore, a pooled analysis for studies with a case-control design was not performed.

The pooled risk ratio for the cohort studies was 1.11, 95% CI [0.93-1.31], z-test was non-significant (p = 0.25). The I^2 value was 0%, indicating minimal inter-study heterogeneity.

3.2.5: Subgroup Analysis: Length of Follow-Up Time

All cohort studies were screened for subgroup analyses stratifying for length of subject follow-up time. Four studies (Brinton et al., 2004; dos Santos Silva et al., 2009; Reigstad et al., 2015; van den Belt-Dusebout et al., 2016) performed such analyses. The study by Brinton et al.

(2004) was not included in the previous analyses as it overlapped with the study population used in the study by Brinton et al. (2014). However, the earlier study stratified subjects on follow-up time whereas the later study did not. Therefore, the earlier study was included in this portion of the analysis. Due to the limited number of studies providing the appropriate subgroup analysis, stratification on type of exposure was not performed.

Subgroup measures of effect were grouped as follows: subjects followed for less than 10 years, subjects followed between 10 and 19 years, and subjects followed for greater than 20 years. Reigstad et al. reported effect measures for subjects followed for 1 to 4 years, 5 to 9 years, and more than 10 years. In this case, the first two strata were pooled into the category of 'subjects followed for less than 10 years', and the latter stratum was grouped in the category of 'subjects followed between 10 and 19 years'. The enrollment period for this study extended from 1983-2002. Follow up occurred in 2010. The study reported only 1,383 subjects were enrolled prior to 1992, representing 8.31% of the study population. Therefore, as only a small portion of the population would have more than 20 years of follow-up, it was deemed appropriate to include this measure of effect in the 'subjects followed between 10 and 19 years' category of our analysis. van den Belt-Dusebout et al. reported effect measures for subjects followed less than 5 years, 5 to 9 years, 10 to 14 years, 15 to 19 years, and more than 20 years. The measures of effect for subjects followed for less that 5 years and for subjects followed between 5 and 9 years were pooled into the category of 'subjects followed for less than 10 years' and the 10 to 14 year and 15 to 19 year strata were pooled into the category of 'subjects followed between 10 and 19 years'. Forest plots for each of these categories are displayed in Figures 3.7, 3.8 and 3.9.

There were two individual effect measures which indicated significant associations. Reigstad et al. found gonadotropin-based stimulation protocols for IVF or ICSI were

significantly associated with breast cancer in women followed for more than 10 years, RR 1.35, 95% CI [1.07-1.71] (Reigstad et al., 2015). dos Santos Silva et al. reported a significant association between clomiphene exposure and breast cancer in women who were followed for more than 20 years, RR 1.65, 95% CI [1.10-2.48] (dos Santos Silva et al., 2009).

The pooled RR for subjects followed less than 10 years was 0.98, 95% CI [0.82-1.17], z-test was non-significant ($p = 0.84$). The I^2 measure indicated moderate levels of heterogeneity, $I^2 = 34\%$. The pooled RR for subjects followed between 10 and 19 years was 1.08, 95% CI [0.97-1.21]. The z-test for this category was non-significant ($p = 0.17$). There was low to moderate inter-study heterogeneity, $I^2 = 27\%$. Finally, the pooled RR for subjects followed for more than 20 years was 1.27, 95% CI [0.91-1.78]. The z-test for this category was non-significant ($p = 0.15$). Inter-study heterogeneity was high, $I^2 = 64\%$.

3.3: Discussion

The meta-analysis failed to find an association between ovarian-stimulating fertility treatments and breast cancer, regardless of type of exposure(s) assessed or the study design. These results are consistent with the findings of two previous meta-analyses synthesizing the literature on this topic (Sergentanis et al., 2014; Zreik et al., 2010). The study by Sergentanis et al. identified IVF as its exposure of interest and the study by Zreik et al. included both clomiphene and gonadotropins as its exposures of interest. Both analyses concluded that there was no evidence of an association between ovarian-stimulating fertility treatments and breast cancer.

The secondary analysis indicated that ovarian stimulation has not been shown to be associated with breast cancer regardless of the length of subject follow-up time. There was some

concern raised in qualitative reviews on this topic stating that definitive conclusions regarding the safety of ovarian stimulation in the context of breast cancer risk cannot be made as previous observational studies had limited follow-up time (Brinton, Moghissi, Scoccia, Westhoff, & Lamb, 2005; Practice Committee of the American Society for Reproductive Medicine, 2016; Stewart & Hart, 2015). Breast cancer is a disease with a relatively long latency period. Estimating the length of this latency period is challenging as tracking tumor development in human subjects poses methodological and ethical issues. The latency period is defined as the time between the onset of the disease to the time where the cancer has grown to a clinically detectable mass. To date, the literature on the topic has failed to definitely quantify the latency period of breast cancer, however there is some evidence supporting the notion that this period is significant. The current observational evidence supporting a long latency period comes from a study which followed women exposed to significant quantities of radiation (Wanebo et al., 1968). The study by Wanebo et al. included 31 women diagnosed with breast cancer after being exposed to the radiation emitted by the nuclear explosions occurring in Hiroshima and Nagasaki in 1945. The study reported the average latency period between exposure to radiation and diagnosis with breast cancer was 15.4 years (Wanebo et al., 1968). It should be noted that it is impossible to conclude that cancers which originate due to a radiation insult progress in the same fashion as those rooted in a hormonal etiology. Nevertheless, it is prudent to study whether lengthened periods of follow-up would reveal a significant association as shorter periods of follow-up may be insufficient in capturing the true effects of the exposure.

There were only two studies included in this meta-analysis which reported significant associations between ovarian-stimulating fertility treatments and breast cancer. Reigstad et al. reported a significant association between gonadotropin use and breast cancer, and Lerner-Geva

et al. reported a significant association between clomiphene use and breast cancer (Lerner-Geva et al., 2006; Reigstad et al., 2015). It is important to note that these studies utilized general population controls. Infertile women are inherently at an increased risk of developing breast cancer for multiple reasons. First, they are more likely to remain nulliparous, have a lower total parity, and have a later age of first birth than the general population, both of which have been shown to be risk factors for breast cancer (Lambe et al., 1996). Additionally, on average, they are expected to have fewer total pregnancies. High total parity has been shown to be protective against breast cancer (Ursin et al., 2004). Finally, many of the lifestyle factors which have an impact on fertility such as alcohol use, smoking, recreational drug use, and a high BMI are also risk factors for breast cancer.

The study by Reigstad et al. utilized a Norwegian birth registry as a source of data. Their entire study population was therefore parous, and age of first birth and total parity were included as covariates in their statistical model. However, lifestyle variables associated with infertility were not included in the model, and the authors correctly identified this as a limitation of their study. This highlights one of the major methodological challenges in attempting to evaluate this relationship of interest. Infertility, and its associated conditions, is an important confounder when evaluating the relationship between ovarian stimulation and breast cancer. Gathering the necessary information to appropriately control for the effects of infertility requires substantial amounts of information frequently not readily available to researchers. Consequently, many studies have opted to restrict their study populations to infertile subjects. There were 7 cohort studies (Brinton et al., 2014; Brinton et al., 2013; dos Santos Silva et al., 2009; Rossing et al., 1996; Stewart et al., 2012; van den Belt-Dusebout et al., 2016; Venn et al., 1999) and 1 case-control study (Jensen et al., 2007) which did so. None of these studies found the existence of a

significant association between ovarian stimulation and breast cancer. Studies also had limitations pertaining to the methods in which they gathered information. These limitations will be discussed in further detail in Chapter 4.

The current body of literatures disputes the postulated association between ovarian-stimulating fertility treatments and breast cancer. While these treatments do indeed raise serum estrogen levels to supraphysiologic levels, they do so only transiently. There is no evidence supporting any sort of long-term impact on the hormone levels of future, natural, ovulatory cycles. Therefore, it is entirely possible that estrogen levels are increased for too transient a period to have any noticeable impact on breast cancer risk. Consequently, perhaps undergoing a greater number of stimulated cycles may increase a woman's risk of breast cancer as this would increase the time period where serum estrogen levels are supraphysiologic. A total of 9 cohort studies (Brinton et al., 2014; Brinton et al., 2013; dos Santos Silva et al., 2009; Orgeas et al., 2009; Pappo et al., 2008; Rossing et al., 1996; K. L. Terry et al., 2006; van den Belt-Dusebout et al., 2016; Venn et al., 1999) and 2 case-control studies conducted subgroup analyses analyzing a potential dose-response relationship (Burkman et al., 2003; Jensen et al., 2007). Unfortunately, there was considerable inter-study heterogeneity pertaining to stratification on the basis of number of cycles, and pooling these studies was not feasible. Only three studies found an association between breast cancer risk and number of ovarian stimulation cycles performed. Orgeas et al. (2009) found the risk of breast cancer was significantly elevated in women who underwent more than 4 cycles of clomiphene treatment (SIR 1.90, 95% CI [1.08-3.35]) (Orgeas et al., 2009). Burkman et al. reported a significant association in subjects undergoing more than 4 cycles of ovarian stimulation using hMG (RR 2.1, 95% CI [1.0-4.4]) (Burkman et al., 2003). It should be noted that both these analyses were limited in terms of number of exposed subjects, as

evidenced by their relatively large standard error for their measure of effect, and their results should be interpreted with caution. Finally, Brinton et al. reported the risk of breast cancer was significantly elevated in subjects undergoing more than 12 cycles of clomiphene therapy (HR 1.45, 95% CI [1.02-2.05]). The remaining studies found no evidence of a dose-response relationship. While it was not assessed quantitatively, evidence supporting any sort of increase in breast cancer risk with increasing number of stimulated cycles is limited.

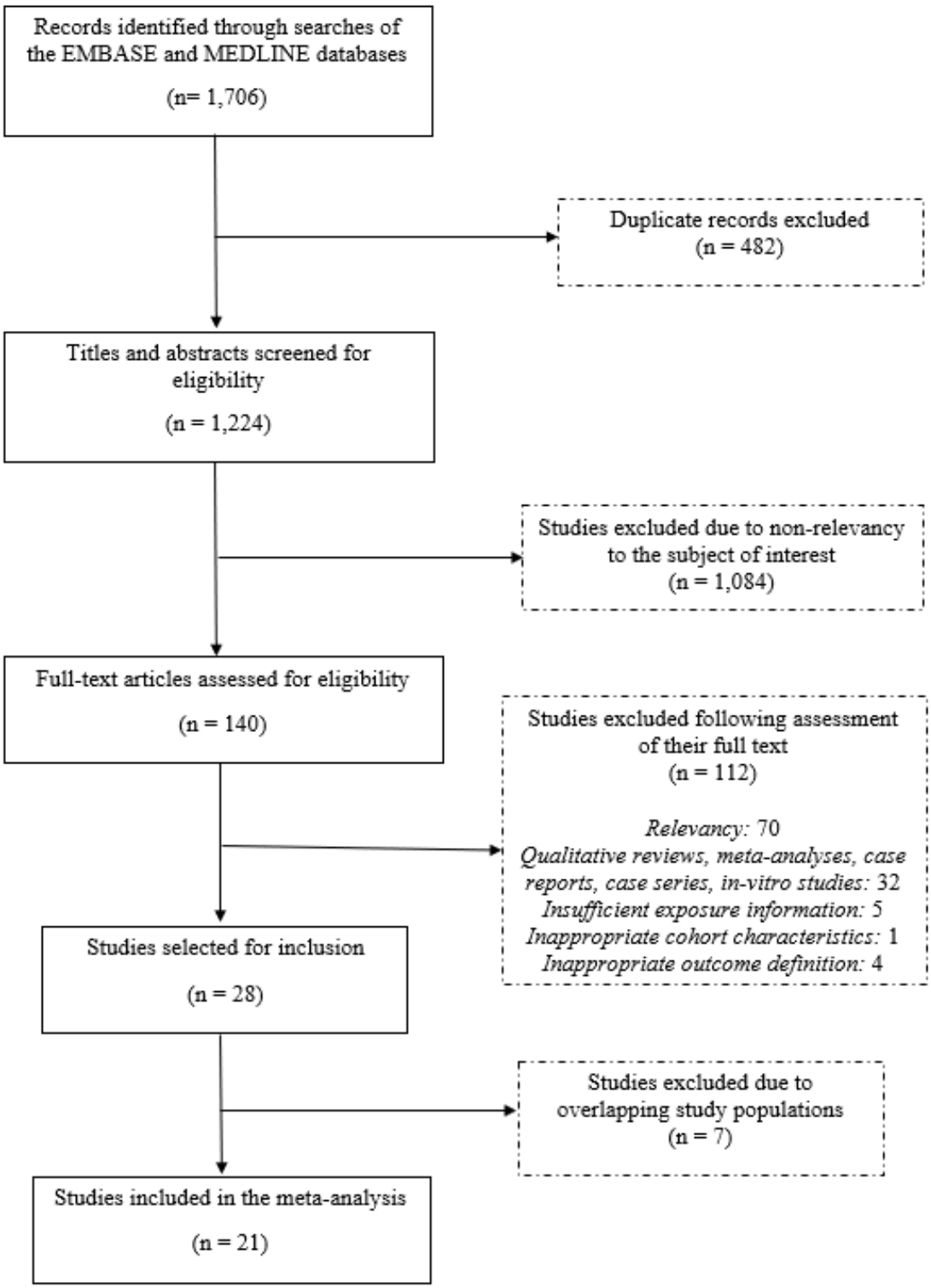

Figure 3.1: Flow Chart – Study Selection

Table 3.1: Characteristics of Included Cohort Studies

Study, year	Location	Cohort Size, description	Enrollment Period, End of Follow-up	Follow-up Time	Exposures Assessed	Types of Controls	Measure of Effect	Covariates Adjusted for in Model
Van den Belt Dusebout, 2016	Netherlands	25,108 women undergoing fertility investigations	1980-1995, follow-up through 2013	Median of 21 years	Gonadotropins	Provided analysis with both general population controls and infertile population controls	HR	Age, age of first birth, number of births
Reigstad, 2015	Norway	806,834 women having given birth	1984-2010, follow-up through 2010	Mean of 16 years	Gonadotropins	Unexposed women from the cohort	HR	Age, calendar period of follow-up, region, parity
Brinton, 2014	United States	9,892 women registered at reproductive endocrinology clinics	1965-1988, follow-up through 2010	Mean of 28.9 years	Clomiphene Gonadotropins	Unexposed women from the cohort	HR	Study site, calendar year of infertility investigation, parity
Brinton, 2013	Israel	87,418 women registered with fertility issues	After 1994 (end of enrolment period not stated),	Mean of 8.1 years	Clomiphene Gonadotropins	Unexposed women from the cohort	HR	Age at entry, BMI, smoking, parity at exit, SES

			follow-up through 2011					
Stewart, 2012	Australia	21,025 women having undergone investigation or treatment for infertility	1983-2002, follow-up through August 2010	Mean of 16 years	Gonadotropins	Unexposed women from the cohort	HR	Age, age at first birth, multiple births
Lerner-Geva, 2012	Israel	2,431 women undergoing infertility investigations	1964-1974, follow-up through 2005	Mean of 34 years	Clomiphene Gonadotropins	General population	SIR	Sex, age, continent of birth
Kallen, 2011	Sweden	1,388,371 giving birth during the study period	1982-2006, end of follow-up not published	Mean of 6.2 years	Gonadotropins	General population	OR	Smoking, age at delivery, year of delivery
Calderon-Margalit, 2009	Israel	15,392 women giving birth in the study period	1964-1976, follow-up through 2004	Mean of 27.6 years	Clomiphene	Unexposed women from the cohort	HR	Age, socioeconomic status, maternal country of birth, BMI
Orgeas, 2009	Sweden	1,135 women treated for infertility at fertility clinics	1961-1976, follow-up through 2004	Mean of 32.1 years	Clomiphene	General population	SIR	Age at end of follow-up, age of first birth, parity, calendar period of diagnosis
dos Santos Silva, 2009	United Kingdom	7,355 women seeking treatment for ovulatory disorders	1963-1999, follow-up through 2005	Mean of 21.4 years	Clomiphene Gonadotropins	Unexposed women from the cohort	RR	Age, calendar period

Pappo, 2008	Israel	3,375 women who underwent IVF	1986-2003, follow-up through 2004	Mean of 8.1 years	Gonadotropins	General population	SIR	Age, continent of birth
Lerner-Geva, 2006	Israel	5,788 women attending fertility clinics	1964-1984, follow-up through 1996	Mean of 20.9 years	Clomiphene Gonadotropins	General population	SIR	Age, place of origin, type of infertility
Terry, 2006	United States	116,671 women aged 25 to 42	1989, follow-up through 2001	Mean of 10.9 years	Clomiphene	Unexposed women from the cohort	HR	Age, height, current BMI, BMI at 18 years old, family history of breast cancer, history of benign breast disease, age of menarche, parity, oral contraceptive use, physical activity
Gauthier, 2004	France	92,555 women enrolled in a national health insurance database	1990-1992, follow-up through June 2000	Mean of 9.7 years	Clomiphene Gonadotropins	Unexposed women from the cohort	RR	Education, smoking, BMI, family history breast cancer, age of menarche, menopausal status, parity, age of first pregnancy
Dor, 2002	Israel	5,026 women treated for infertility at fertility clinics	1981-1992, follow up through 1996	Mean of 3.63 years	Clomiphene Gonadotropins	General population	SIR	Age, sex, place of birth
Venn, 1999	Australia	29,700 women registered at fertility clinics	Prior to 1994, follow-up through 1996	Mean of 8.5 years	Gonadotropins	Unexposed women from the cohort	SIR	Age, calendar period, national region

Rossing, 1996	United States	3,837 women seeking fertility counselling	1974-1986, follow-up through 1991	Mean of 11.32 years	Clomiphene	Unexposed women from the cohort	RR	Age, weight at cohort entry, calendar year at entry

Table 3.2: Characteristics of the Included Case-Control Studies

Study, year	Location	Enrollment Period	Exposures Assessed	Number of Cases, Number of Controls	Type of Controls	Measure of Effect	Covariates Adjusted for in Model
Yli-Kuha, 2012	Finland	1996-2004	Gonadotropins	55 cases 60 controls	General population	OR	Age, geographic location, socioeconomic status, marital status
Fei, 2012	United States	2008-2010	Clomiphene Gonadotropins	1,669 cases 1,422 controls	Sisters of cases	OR	Birth order of sisters, menopausal status at index, age of first birth
Jensen, 2007	Denmark	1965-1998	Clomiphene Gonadotropins	331 cases 1,226 controls	Infertile controls	RR	Age, parity, number of births
Burkman, 2003	United States	1994-1998	Clomiphene Gonadotropins	4,566 cases 4,676 controls	General population	OR	Age, ethnicity, study site

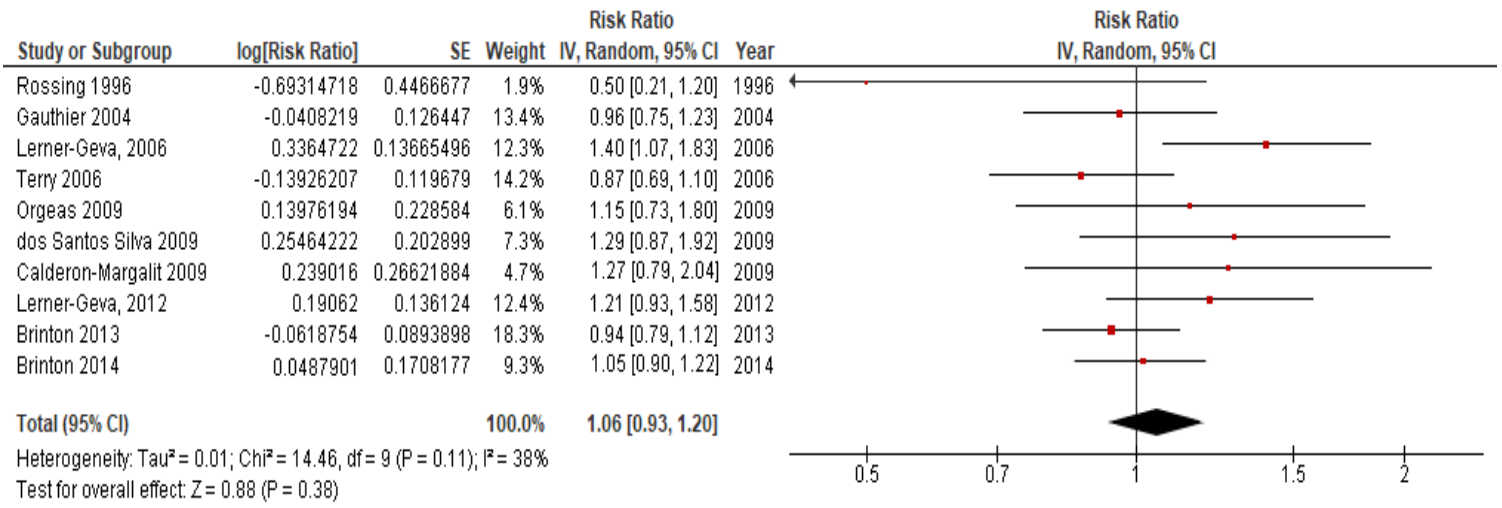

Figure 3.2: Forest plot and pooled RR – All cohort studies examining the association between clomiphene and breast cancer included in the analysis

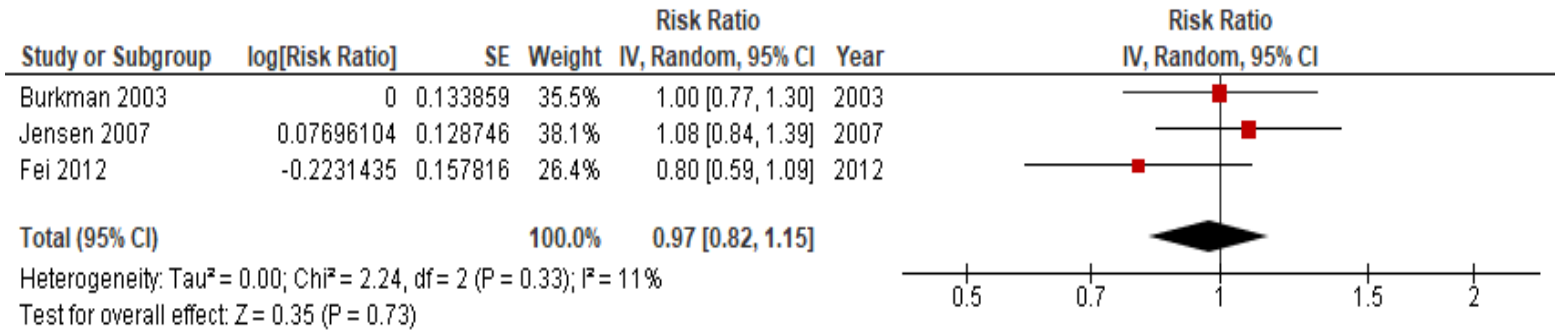

Figure 3.3: Forest plot and pooled RR – All case-control studies examining the association between clomiphene and breast cancer included in the analysis

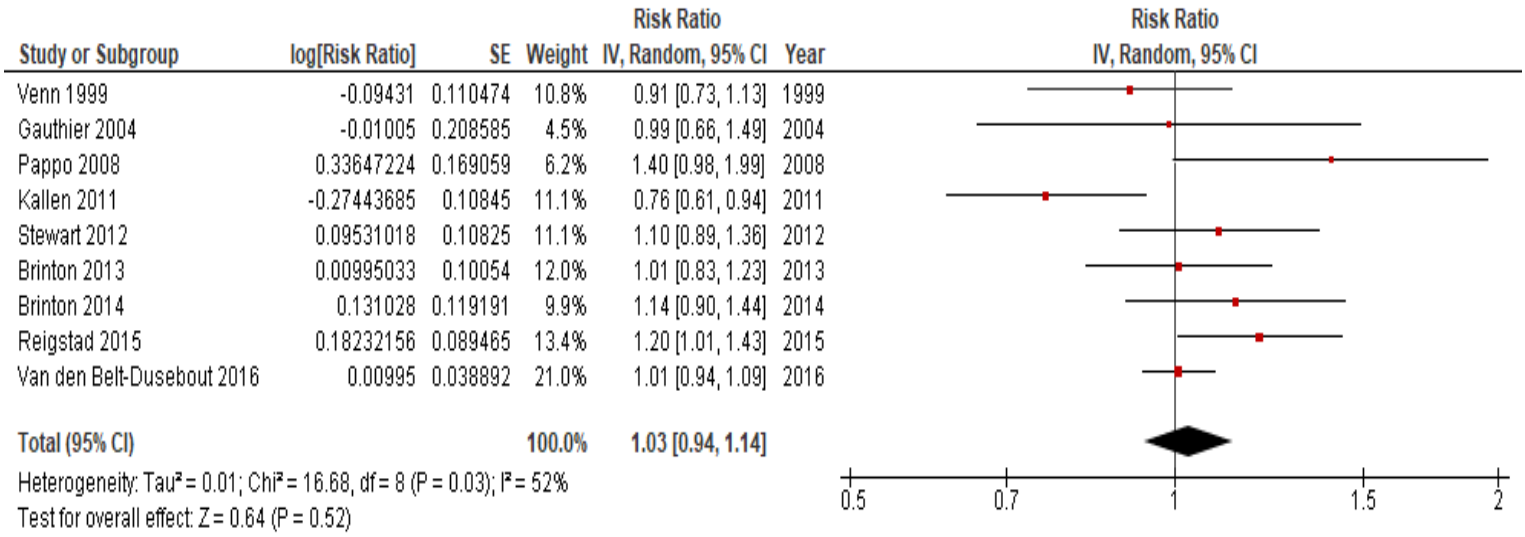

Figure 3.4: Forest plot and pooled RR – All cohort studies examining the association between gonadotropins and breast cancer included in the analysis

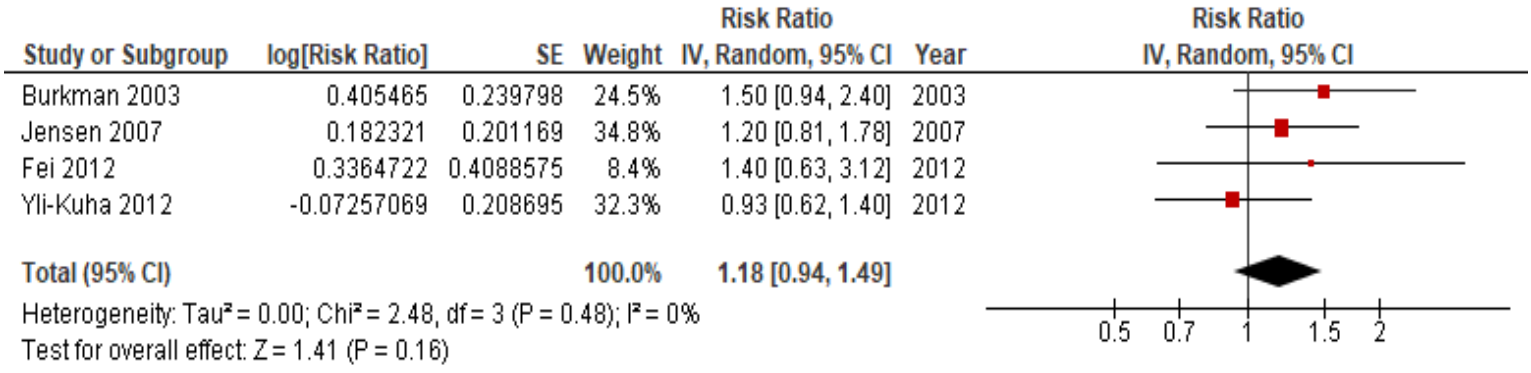

Figure 3.5: Forest plot and pooled RR – All case-control studies examining the association between gonadotropins and breast cancer included in the analysis

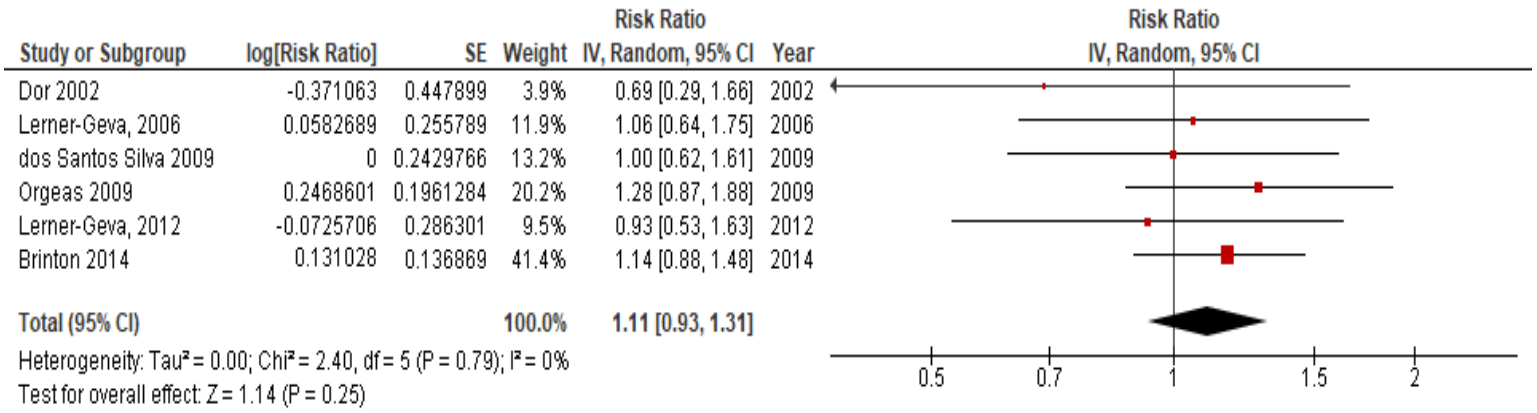

Figure 3.6: Forest plot and pooled RR – All cohort studies examining the association between clomiphene plus gonadotropins and breast cancer included in the analysis

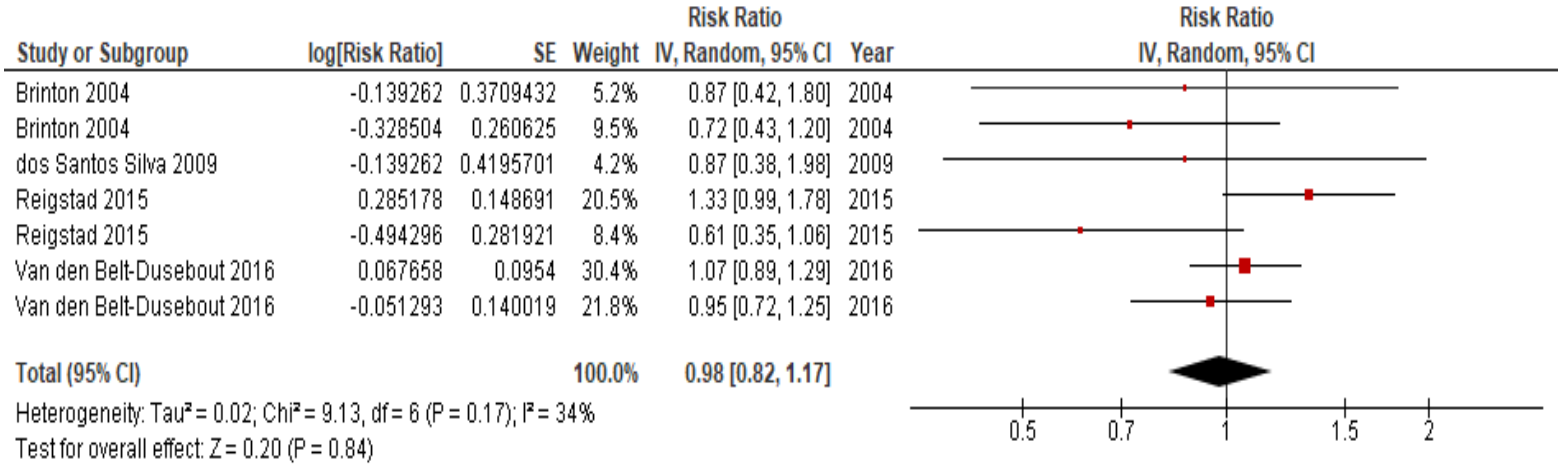

Figure 3.7: Forest plot and pooled RR – Cohort studies which conducted a stratified analysis on follow-up time, measures of effect for subjects followed less than 10 years

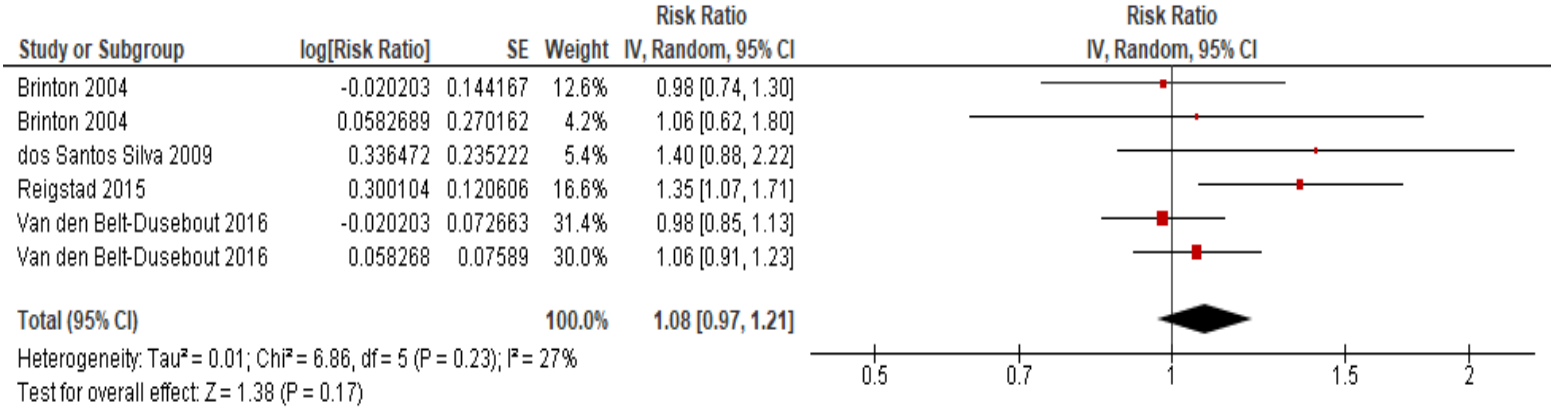

Figure 3.8: Forest plot and pooled RR – Cohort studies which conducted a stratified analysis on follow-up time, measures of effect for subjects followed between 10 to 19 years

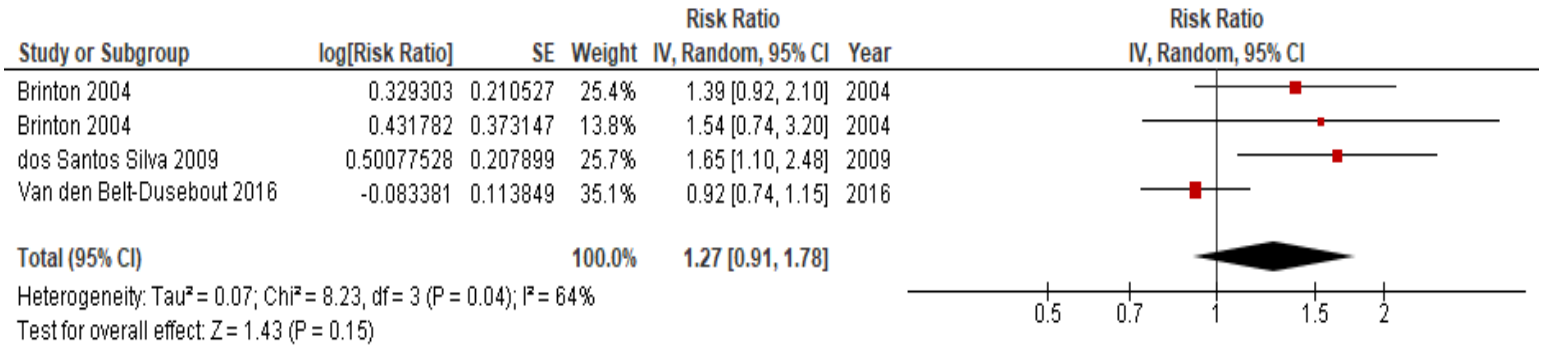

Figure 3.9: Forest plot and pooled RR – Cohort studies which conducted a stratified analysis on follow-up time, measures of effect for subjects followed more than 20 years

Chapter 4: Case-Control Study

A case-control study was performed to examine the association between ovarian stimulation and breast cancer utilizing the Clinical Practice Research Datalink (CPRD). The protocol for this study was approved by the Independent Scientific Advisory Committee (ISAC) in March of 2017 (protocol number: 17_043). The following chapter contains a manuscript detailing the materials, methods, and results of this study, as well as a discussion of the results. To limit redundancy with previous chapters the introduction of this manuscript was removed as it contained similar information to that presented in Chapters 1 and 2.

Ovarian-Stimulating Fertility Treatments and the Long-Term Risk of Breast Cancer: A Case-Control Study Using the Clinical Practice Research Datalink (CPRD)

Adriano Petrangelo, BSc[1]

Nicholas Czuzoj-Shulman, MMA[2]

Haim A. Abenhaim, MD, MPH [1, 2]

Montréal, Québec, Canada

[1] Department of Obstetrics and Gynecology, Jewish General Hospital, McGill University, Montréal, Québec, Canada

[2] Centre for Clinical Epidemiology and Community Studies, Jewish General Hospital, Montréal, Québec, Canada

No funding was received for this study.

Authors have no conflicts of interest to report.

Corresponding Author:

Haim A. Abenhaim, MD, MPH, FRCSC

Jewish General Hospital, Obstetrics & Gynecology, McGill University

Pavilion H, Room 325

5790 Cote-Des-Neiges Road,

Montréal, Québec, H3S 1Y9

Tel: 514-340-8222 x5488

Fax: 514-340-7941

Email: haim.abenhaim@gmail.com

Abstract

Introduction: The interplay between reproductive hormone levels and breast cancer is well established. Ovarian-stimulating fertility treatments raise serum estrogen levels transiently over the course of therapy, which has raised questions regarding their safety.

Methods: A population-based case-control study was performed using the Clinical Practice Research Datalink. Cases of breast cancer diagnosed between 1995 and 2013 were matched to 10 controls based on age, general practice, and follow-up time. Cases and controls were assessed for exposure to clomiphene or IVF via their medical records. Risk ratios and their 95% confidence intervals were computed using multivariate logistic regression. The model was adjusted for BMI, smoking, alcohol use, parity, hormonal contraceptive use, hormone replacement therapy, and oophorectomy.

Results: The study population consisted of 27,026 cases of breast cancer and their controls. 1,717 subjects had a history of clomiphene exposure and 1,137 subjects had a history of IVF. Exposure to clomiphene was significantly associated with breast cancer, RR 1.32, 95% CI [1.23-1.42]. Exposure to IVF was also associated with breast cancer, RR 1.55, 95% CI [1.42-1.69]. The stratified analysis indicated clomiphene was associated with pre-menopausal cancers, RR 1.58 95% CI [1.45-1.72] but not post-menopausal malignancies, RR 0.91 95% CI [0.80-1.03]. IVF was associated with both pre- and post-menopausal cancers. A secondary analysis stratified the study population on PCOS status. Among women with diagnoses of PCOS, clomiphene use was not associated with an increased risk of breast cancer, RR 1.22, 95% CI [0.99-1.50].

Conclusion: Cases of breast cancer were significantly more likely to have underwent clomiphene or IVF treatment. However, underlying infertility and its associated conditions may have confounded this relationship and these results should be interpreted with caution.

4.1 Materials and Methods

We performed a population-based case-control study using the Clinical Practice Research Datalink (CPRD). The CPRD is a primary care database collecting information from over 674 general practices in the United Kingdom, including medical records for over 11.3 million patients (Herrett et al., 2015). As of June 2015, there were an estimated 4.4 million patients registered in the database, representing approximately 7% of the UK's population (Herrett et al., 2015). Demographics of the CPRD's population have been compared to information collected by the UK's 2011 census. The population captured within the CPRD was deemed to be broadly representative of the general population in terms of age, sex, and ethnicity (Mathur et al., 2014). The CPRD is maintained by the UK's Department of Health in association with the National Institute for Health Research. These regulatory bodies have instituted quality assurance and compliance regulations to ensure accuracy of the recorded information. General practices are individually evaluated and approved. Quality assurance for medical records is performed in accordance with the Quality and Outcomes Framework, a guideline outlining the reporting standards for general practitioners in the UK (Sutcliffe D, 2012). The use of the CPRD is advantageous in epidemiological studies, particularly those evaluating etiological causes of cancer, due to its longitudinal nature. Data collection occurs prospectively, thereby reducing the risk of recall bias. Additionally, as breast cancer is a disease with a relatively long latency period, extended follow-up times are generally preferred in studies evaluating its causal risk factors (Wanebo et al., 1968). The CPRD includes patient consultation records from 1987 onwards, with active patients having a median follow-up time of 9.4 years (Herrett et al., 2015).

A protocol for this study was submitted to the Independent Scientific Advisory Committee (ISAC) for review. The ISAC is an advisory body evaluating the feasibility,

ethicality, confidentiality, quality, and value of studies vying to use CPRD information. The protocol was approved by the Committee in March of 2017 (Protocol number 17_043).

4.1.1: Study Population and Design

The source population from which both cases and controls were selected was defined as all females with records present in the CPRD between January 1st, 1995 and January 1st, 2015. A subject entered the source population at the occurrence of the latest of the following events: registration with an up-to-standard general practice as of January 1st, 1995, calendar date of the subject's 18th birthday, or date of first registration with an up-to-standard general practice. Up-to-standard practices are designated as such following quality assurance reviews performed by the regulatory bodies maintaining the CPRD (Reeves et al., 2014). Subjects were excluded from the source population if they were not from an up-to-standard general practice, had less than one year of medical history recorded in the CPRD, or were diagnosed with any form of cancer prior to a first diagnosis of breast cancer. The latter exclusion criterion was used to ensure all cases of breast cancer were not re-occurrences of a prior breast malignancy or a metastasis of any other malignancy. The source population was restricted to females born between January 1st, 1945 and January 1st, 1995. The left censoring date ensured no female in the source population would be older than the age of 50 as of January 1st, 1995. As a result, all subjects entering the source population on January 1st, 1995 had the possibility of being exposed to fertility treatments and having these treatments captured within the CPRD. The right censoring date ensured no female in the source population will be younger than 18 years of age as of January 1st, 2013. The two-year disparity between the right censoring date and the end of the study period allowed for a minimum of two years of follow-up following the subject's 18th birthday.

4.1.2: Case and Control Selection

The entire source population was followed until a first diagnosis of breast cancer, death from any cause, end of registration with the general practice, or end of the study period, whichever occurred first. Diagnoses of breast cancer were identified with the use of a validated computer algorithm used in previous studies performed with CPRD records (Opatrny, Dell'Aniello, Assouline, & Suissa, 2008). The list of medical and read codes used in outcome identification is found in the appendix.

For each case of breast cancer identified, 10 controls were selected. Control subjects were matched with their respective cases based on year of birth, general practice, and year of registration in the general practice. Thus, cases and controls were matched on age, general practice, and follow-up time. All controls were alive, never diagnosed with breast cancer, and registered with an up-to-standard general practice when matched to their corresponding case. The index date for cases was used as the index date for their matched controls. All baseline variables were evaluated as of the index date for cases and controls. The choice of 10 controls per case allowed the analysis to be manageable, with minimal loss of precision (Breslow NE, 1987).

4.1.3: Exposure Assessment

All cases and controls were evaluated for exposure to either clomiphene or IVF. Medical and read code lists were generated by searching the CPRD Code Browser with the relevant search terms. A list of codes used to identify cases of exposure is found in the appendix. As the CPRD contains primary care records, evaluation of the types and dosages of the drugs used in IVF treatments was not possible, as this information is restricted to the records of secondary or tertiary centres. Evaluating IVF exposure on an ‘ever/never’ basis was still feasible as IVF

treatments are still likely to be recorded in a patient's primary care file, either due to the patient being referred to a fertility clinic by the primary care physician, or the patient reporting these treatments to their primary care physician. Conversely, it is not uncommon for clomiphene to be prescribed to patients by primary care physicians in the UK. NICE guidelines published in 2004 and updated in 2013 recommend physicians "offer ultrasound monitoring during at least the first cycle of treatment to ensure [patients] are taking a dose that minimises the risk of multiple pregnancy" (National Collaborating Centre for Women's and Children's Health, 2013). These guidelines would certainly dissuade some primary care physicians from providing clomiphene treatment. Some authors argue ultrasound monitoring for clomiphene treatment is not necessary as ultrasound monitoring has not been shown to consistently decrease the risk of multiple pregnancy following clomiphene use (Cahill & Wardle, 2002). Studies have shown that some physicians in the UK still prescribe clomiphene in the primary care setting (Wilkes, Chinn, Murdoch, & Rubin, 2009). It should be noted, however, that a sizeable portion of females undergoing clomiphene treatment would do so in specialized fertility clinics. The use of primary care data to evaluate exposure to either clomiphene or IVF is therefore an important limitation of our study.

4.1.4: Statistical Analysis

First, descriptive statistics were used to summarize the characteristics of cases and their matched controls. Second, multivariate logistic regression was used to estimate RRs and their 95% confidence intervals. As we matched cases and controls on follow-up time, odds ratios (ORs) computed by the regression model were reliable estimators of the RRs. The regression model was adjusted for a series of covariates considered to be potential confounders of the relationship between ovarian-stimulating fertility treatments and breast cancer. These variables

were body mass index (BMI), smoking, alcohol use, parity, hormonal contraceptives use, hormone replacement therapy (HRT), and oophorectomy. BMI values were stratified into four categories in accordance with WHO definitions, that is, Underweight (BMI <18.50 kg/m^2), Normal (BMI 18.50-24.99 kg/m^2), Overweight (BMI 25.00-29.99 kg/m^2), and Obese (BMI ≥30.00 kg/m^2) (Nuttall, 2015). Smoking status was stratified into never use, current light use, current moderate use, current heavy use, and ex-heavy use. Alcohol use was stratified into similar categories. Both hormonal contraceptive use and HRT use were evaluated on an 'ever/never' basis. Hormonal contraceptives included combined oral contraceptives, progestin-only pills (POPs), and implantable progestin devices. POPs and implantable progestin devices were included due to the evidence presented in the paper by Morch et al. which found a significant association between these medications and breast cancer (Morch et al., 2017). Finally, oophorectomy was evaluated as a binary, 'yes/no', variable. Instances of oophorectomy must have occurred prior to a subject's index date. Lists of CPRD read codes for these variables can be found in the appendix.

Our primary analysis consisted of evaluating clomiphene and IVF exposure on an 'ever/never' basis. Additionally, cases of breast cancer were stratified on age of diagnosis, that is, whether the diagnosis occurred before the age of 50 or after the age of 50. This approximates whether the breast cancer was pre-menopausal or post-menopausal.

Two secondary analyses were performed. The first aimed to determine the possible relationship between the number of clomiphene pills prescribed and the risk of breast cancer. This analysis evaluated the potential dose-response relationship between clomiphene usage and breast cancer. Secondly, as we were unable to adjust for underlying infertility and some of its associated conditions such as total parity and age of first birth, we aimed to evaluate the potential

confounding effects of these variables in a different manner. This involved stratifying the study population on previous diagnoses of polycystic ovary syndrome (PCOS). Subjects were identified as having PCOS if their clinical records indicated they had been diagnosed with this syndrome prior to their date of exposure to either clomiphene or IVF. The list of read codes used to identify subjects diagnosed with PCOS can be found in the appendix of this document.

In order to address the issue of missing data, we used multiple imputation methods that have been shown to produce valid estimates of effect when the rate of missing data is less than 60% (Barzi & Woodward, 2004). Multiple imputation of missing values was performed using the SAS function PROC MI on all covariate model variables to produce the values for imputation. Similar to a study by Delaney et al. which successfully used this technique in a study using the CPRD, we used mixed chain imputation with 1000 burn-in iterations and the Markov chain Monte Carlo (MCMC) method in which a Markov chain long enough for the distribution of the elements to stabilize to a common, stationary distribution (Delaney et al., 2007). By repeatedly simulating steps of the chain, draws from the distribution of interest are also simulated. In keeping with the Delaney et al. methodology, time-plots and auto-regression plots were assessed to determine whether imputation was successful and that there was minimal auto-regression between iterations of the MCMC algorithm. The results for each imputation were generated using conditional logistic regression and then combined using PROC MIANALYZE procedure in SAS. Finally, 10 imputed datasets for this study were produced to ensure that our effect estimates were not overly inaccurate due to Monte Carlo variability (Schafer, 1999). All analyses were done with the statistical software package SAS Institute Inc. Enterprise Guide 6.1, Copyright ©2013. SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

4.2: Results

The study population consisted of 27,026 cases of breast cancer and their corresponding 270,260 matched controls. Covariate characteristics for the study population, including demographic, lifestyle, and clinical variables, are displayed in Table 4.1. Breast cancer cases were more likely to be underweight (BMI of less than 18.5 kg/m^2), to have been prescribed hormonal contraceptives, to have been prescribed HRT, and be parous. Cases and controls were similar in terms of smoking status, alcohol use, and rates of previous oophorectomy.

The results from the primary analysis are presented in Table 4.2. In total, 1,717 subjects were exposed to clomiphene and 1,137 underwent IVF treatment. Following adjustment for the relevant covariates, exposure to clomiphene was significantly associated with breast cancer, RR 1.32, 95% CI [1.23-1.42]. Similarly, exposure to IVF was significantly associated with breast cancer, RR 1.55, 95% CI [1.42-1.69]. Following stratification based on age of breast cancer diagnosis, exposure to clomiphene remained significantly associated to pre-menopausal breast cancers, RR 1.58, 95% CI [1.45-1.72], but not post-menopausal breast malignancies, RR 0.91, 95% CI [0.80-1.03]. IVF remained significantly associated to both pre-menopausal and post-menopausal breast cancers with RRs of 1.61, 95% CI [1.44-1.79] and 1.43, 95% CI [1.25-1.64], respectively.

Table 4.3 presents the results of the first secondary analysis exploring the dose-response relationship between clomiphene and breast cancer. Clomiphene remained associated with breast cancer regardless of the number of pills prescribed, except in subjects who were prescribed between 46 and 60 pills, RR 1.13, 95% CI [0.93-1.38]. No apparent trend existed. The second secondary analysis stratified cases and controls based on prior diagnoses of PCOS. The results of this analysis are displayed in Table 4.4. A total of 2,264 women were diagnosed with PCOS, 203

of which were prescribed clomiphene. In women without PCOS, clomiphene exposure remained significantly associated with breast cancer, RR 1.24, 95% CI [1.16-1.33]. In women who were diagnosed with PCOS, clomiphene use was not significantly associated with an increased risk of breast cancer, RR 1.22, 95% CI [0.99-1.50].

4.4: Discussion

Determining whether ovarian-stimulating fertility treatments are linked with breast cancer is clinically important. Evidence supporting this relationship would potentially modify treatment protocols for future patients and would justify more robust screening regimens for patients having already undergone ovarian stimulation. Numerous observational studies have explored this relationship. The results of these studies have been synthesized in the meta-analysis presented in Chapter 3. Overall, the current evidence on this topic refutes any relationship between ovarian stimulation and breast cancer, however, many previous studies suffered from certain limitations which we aimed to improve upon.

Firstly, several previous observational studies utilized multiple, distinct databases to compile information. This requires the use of data linkage. For example, studies such as the ones by van den Belt-Dusebout et al., Brinton et al., and Stewart et al. ascertained exposure information from the medical records of fertility clinics (Brinton et al., 2014; Stewart et al., 2012; van den Belt-Dusebout et al., 2016). At follow-up, subjects were linked to national cancer registries to determine their outcome status. Data linkage is a common tool used in epidemiological studies, however, it is susceptible to bias. When databases are linked, errors may occur. These errors are often due to missing data, leading to missed matches, or erroneous patient identifiers in one of the linked databases, leading to false matches (Harron, Wade, Gilbert, Muller-Pebody, & Goldstein, 2014). Linkage bias stems from missed or erroneous matching

occurring more often when linking subjects with certain characteristics. Bohensky et al. conducted a systematic review of the literature quantitatively evaluating linkage bias, mainly by comparing the characteristics of correctly matched and falsely matched records (Bohensky et al., 2010). Of the 13 studies included in the analysis, 11 if them identified instances of bias arising from data linkage. These biases were primarily the result of disparities in overall health, age, geographical location, ethnicity, time period of enrollment, and socioeconomic status between matched and unmatched, or erroneously-matched, subjects. In our study, exposure, outcome, and covariate information was extracted from a single database which effectively eliminated the risk of bias arising from data linkage. A second major source of bias in previous studies arose from the methods of gathering covariate information. Cancer registries and fertility clinic records often do not record the necessary information required for appropriate control of confounding. For example, these databases are less likely to include information pertaining to smoking status, alcohol use, HRT use, and hormonal contraceptive use when compared to primary care records. Therefore, studies would either fail to include these covariates in their model or gather information on these variables using patient-filled questionnaires at the time of subject follow-up. This was the case for studies such as van den Belt-Dusebout et al., Burkman et al., and Gauthier et al (Burkman et al., 2003; Gauthier et al., 2004; van den Belt-Dusebout et al., 2016). Gathering covariate information at the time of follow-up causes this information to be susceptible to recall bias. Recall bias, in this context, stems from subjects being more likely to report variables associated with breast cancer should they have been diagnosed with breast cancer in the past. For example, subjects with a history breast cancer would be more likely to report past HRT use compared with those not diagnosed with breast cancer. Following adjustment, this would dilute the association, if any, between the exposure of interest and breast cancer. Our study used information collected by primary care physicians in accordance with

rigorous quality assurance standards, which somewhat increases the validity of the information used to evaluate exposure, outcome, and covariate variables. Information was collected longitudinally, thereby eliminating the possibility of recall bias affecting results. Finally, with 27,026 cases of breast cancer included in the analysis, 211 of whom were exposed to clomiphene and 152 exposed to IVF, our study was substantially larger and more statistically powerful than prior case-control studies. The next-largest case-control study was the one conducted by Burkman et al., which included 61 cases of breast cancer exposed to gonadotropins and 111 cases exposed to clomiphene (Burkman et al., 2003).

Our study had important limitations which warrant further discussion. One such limitation was our inability to control for the effects of infertility and some of its associated factors in the regression model. It is postulated that women who experience fertility issues are inherently at a greater risk of developing breast cancer. Typically, infertile women are more likely to remain nulliparous and give birth later in life in comparison to women with normal reproductive functioning. In cases where they succeed in conceiving, they are likely to have, on average, a lower total parity compared to women without fertility issues. Nulliparity and an older age of first birth are both considered risk factors for developing breast cancer (Lambe et al., 1996). A lower total parity is also associated with an increased risk of breast cancer (National Cancer, 2005). Factors such as alcohol use, smoking, and an elevated BMI are associated with reduced fecundity. Breast cancer is also strongly linked with alcohol use (McDonald, Goyal, & Terry, 2013), smoking (Jones, Schoemaker, Wright, Ashworth, & Swerdlow, 2017) and obesity (Ligibel, 2011), making these factors important confounding variables. In our primary analysis, we attempted to control for the effects of alcohol use, smoking, BMI, and parity, however, we were unable to adjust for age of first birth and total number of pregnancies, which was another important limitation of our study.

In ideal conditions, the entire study population would have been restricted to women with a history of fertility issues as to negate the confounding effects of infertility. This was not feasible as codes for infertility within the CPRD are not standardized and have not been externally validated. Instead, we decided to perform a secondary analysis which stratified the study population on a variable which has been validated and used in previous observational studies using the CPRD, PCOS (Rees, Jenkins-Jones, & Morgan, 2016). This syndrome is a common cause of infertility and is strongly associated with breast cancer risk factors such as obesity (Sam, 2007). Women suffering from PCOS are also be more likely to be nulliparous (Mikola, Hiilesmaa, Halttunen, Suhonen, & Tiitinen, 2001). Within the group of women who were diagnosed with PCOS, the association between clomiphene and breast cancer was not significant. In other words, within a population of women affected by fertility issues, clomiphene was no longer significantly associated with breast cancer. This finding puts into question the whether the significant association observed in the primary analysis was due to a causal relationship between ovarian stimulation and breast cancer as it is possible that these results were confounded by the underlying infertility.

Another limitation of our study was the use of primary care data to evaluate exposure information. Firstly, IVF is performed in secondary and tertiary care centres specialized in providing these treatments. Thus, there is a strong possibility that a significant numbers of exposure events were missed as exposure information was extracted from primary care records. IVF exposure would only be present in the CPRD if the primary care physician referred their patient to a specialty clinic, or the patient self-reported their treatment to their doctor. However, we cannot assume that the misclassification of IVF exposure data was biased in any way. The evaluation of exposure to IVF would be biased if it was more likely to be reported in women with breast cancer than in control subjects. In this case, information on IVF exposure could not

be biased as information contained in the CPRD is collected longitudinally. The use of primary care data also limited our study by preventing an analysis of the dose-response relationship between number of IVF cycles and breast cancer. Information the number of IVF cycles given to each subject would be contained in the records of the fertility clinics these women attended. Additionally, while it is not uncommon for clomiphene treatment to be prescribed by primary care physicians, regional guidelines for the UK suggest patient response to the first cycle of clomiphene treatment be monitored by transvaginal ultrasound (National Collaborating Centre for Women's and Children's Health (UK), 2013). This type of monitoring is often performed in specialized care centres. Therefore, it is likely that a sizeable portion of clomiphene exposures were misclassified. However, similar to the case of IVF, it is unlikely that this misclassification was biased in any way.

Determining whether the observed association between ovarian stimulation and breast cancer is a causal relationship is of important clinical significance. According to the Bradford-Hill considerations for causality, evidence of a dose-response relationship is important for stating a relationship is causal (Hofler, 2005). This was analyzed in the first of the secondary analyses. No apparent trend was seen between the number of clomiphene pills prescribed and the risk of breast cancer. This disputes any sort of dose-response relationship between clomiphene and breast cancer. There is a plausible alternate explanation for this finding. Information on the number of clomiphene pills administered to each patient was extracted from the primary care clinic's prescription records. Stratification was therefore performed based on the number of clomiphene pills prescribed by the subject's primary care physician. It is possible that a sizeable portion of the population was not administered the entire dose of clomiphene prescribed. This may be especially true for subjects prescribed a large quantity of pills. As a result, the association between clomiphene and breast cancer may have been diluted in the higher-dosage strata. This

may have effectively masked a potential dose-response relationship. Nevertheless, these results cannot support a dose-response relationship between clomiphene and breast cancer, which further limits our ability to conclude the existence of a causal relationship between ovarian stimulation and breast cancer.

The possibility that surveillance bias affected results should also be emphasized. Surveillance bias is a form of non-differential bias caused by a group of subjects being clinically monitored more closely than the reference group (Hemminki et al., 2017). In this case, women who require fertility treatments to conceive are be more likely to have co-morbid conditions which were direct or indirect causes of their infertility. In turn, these conditions would cause a subject to require more frequent, and more extensive, medical visits. Conditions such as endocrine dysfunction, endometriosis, PCOS, and sexually transmitted infections may cause a woman to visit their physician more frequently than the general population. Additionally, several lifestyle factors such as alcohol use, smoking, and obesity are associated with other co-morbidities which would necessitate frequent clinical care. Should infertile women visit their physician more often than the general population, breast cancer may be preferentially detected in this group of women, which would bias the association between ovarian-stimulating fertility treatments and breast cancer towards significance.

The disparity is socioeconomic status between the exposed and reference groups may also contribute to surveillance bias. In the UK, IVF treatments are subsidized by the National Health Service (NHS), however, a comprehensive evaluation of each patient is performed to evaluate whether the service will be covered in full. Variables such as age, comorbidities affecting prognosis, and previous births are considered when deciding coverage. Approximately 40% of IVF cycles performed in the UK are fully funded by the NHS. The remaining cycles are privately funded, either partially or fully (Human Fertilisation and Embryology Authority, 2016).

The cost of an IVF cycle may be prohibitively expensive to couples of lower socioeconomic statuses. Therefore, it may be assumed that a sizeable portion of women undergoing IVF are of moderate to high socioeconomic status. Although the UK has a single-payer health care system, low socioeconomic status is still considered to be an important barrier to accessing regular medical care. Observational studies performed in countries utilizing a single-payer health care system consistently conclude that low socioeconomic status is associated with a decreased utilization of clinical services (Filc, Davidovich, Novack, & Balicer, 2014; Olah, Gaisano, & Hwang, 2013). Women of low socioeconomic status have also been shown to be less likely to undergo regular mammographic screening in single-payer systems (Hanson, Montgomery, Bakker, & Conlon, 2009). As it may be assumed that a sizeable portion of women undergoing IVF are of moderate to high socioeconomic status, breast cancers may be preferentially detected in this group, which would further bias results towards significance.

In summary, the results of our study contradict many of the previous analyses examining this relationship, as both clomiphene and IVF was related to an increased risk of breast cancer, even after adjustment for several important confounders. However, we are hesitant to promote the presence of a causal association between ovarian-stimulating fertility treatments and breast cancer. The results our secondary analyses indicate there is a possibility that the results from our primary analysis may have been confounded by the effects of infertility. These results may also have been affected by surveillance bias and confounding due to socioeconomic status. Additionally, a clear dose-response relationship between clomiphene and breast cancer could not be established which further puts into question the existence of a causal relationship between ovarian stimulation and breast cancer.

Table 4.1: Demographic, Clinical, and Lifestyle Characteristics of Breast Cancer Cases and Matched Controls

Characteristics	Breast Cancer *n* =27,026 (%)	Controls *n* = 270,260
Age (years)		
<35	629 (2.33)	6,290 (2.33)
35-49	8,776 (32.47)	87,760 (32.47)
50-64	15,826 (58.56)	158,260 (58.56)
65+	1,795 (6.64)	17,950 (6.64)
BMI (at index)		
<18.50	3,062 (19.23)	51,970 (11.33)
18.50-24.99	10,534 (37.65)	3,062 (38.98)
25.00-29.99	7,378 (24.33)	65,473 (27.3)
30.00+	6,052 (18.90)	51,074 (22.39)
Smoking		
Non-smoker	17,584 (57.27)	154,770 (65.06)
Light-moderate smoker	2,107 (6.82)	18,438 (7.8)
Heavy smoker	710 (2.44)	6,592 (2.63)
Ex-heavy smoker	235 (0.63)	1,694 (0.87)
Missing	6,390 (32.84)	88,766 (23.64)
Alcohol		
Non-drinker	4,310 (16.17)	43,690 (15.95)
Light-moderate drinker	10,122 (31.25)	84,457 (37.45)
Heavy drinker	269 (0.82)	2,212 (1.00)
Ex-heavy drinker	8 (0.04)	117 (0.03)
Missing	12,317 (51.72)	139,784 (45.57)
Parity		
Parous	11,473 (42.45)	93,390 (34.59)
Nulliparous/Missing	15,553 (57.55)	176,870 (65.41)

Hormonal Contraceptive Use		
Ever	7,469 (27.64)	60,454 (22.37)
Never	19,557 (72.36)	209,806 (77.63)
Hormone Replacement Therapy Use		
Ever	4,551 (16.84)	39,897 (14.76)
Never	22,475 (83.16)	230,363 (85.24)
Oophorectomy		
Yes	69 (0.26)	616 (0.23)
No	26,957 (99.74)	269,644 (99.77)

Table 4.2: Overall Association Between Ovarian-Stimulating Fertility Treatments and Breast Cancer

Variable	Cases of breast cancer (n = 27,026)	Controls (n = 270,260)	Crude RR (95% CI)	Adjusted RR[a] (95% CI)	Adjusted *p*-value
Overall					
Clomiphene (n = 1,717)	211	1,506	1.41 (1.32-1.51)	1.32 (1.23-1.42)	<0.0001
IVF (n = 1,137)	152	985	1.55 (1.43-1.68)	1.55 (1.42-1.69)	<0.0001
Age of breast cancer diagnosis - Clomiphene					
Diagnosis before 50	154	944	1.65 (1.52-1.79)	1.58 (1.45-1.72)	<0.0001
Diagnosis after 50	57	562	1.01 (0.90-1.14)	0.91 (0.80-1.03)	0.1400
Age of breast cancer diagnosis - IVF					
Diagnosis before 50	95	585	1.63 (1.47-1.81)	1.61 (1.44-1.79)	<0.0001
Diagnosis after 50	57	400	1.43 (1.26-1.62)	1.43 (1.25-1.64)	<0.0001

[a] Adjusted for BMI, smoking, alcohol use, parity, hormonal contraceptive use, HRT use and oophorectomy

Table 4.3: Association between Clomiphene and Breast Cancer - Stratified by Number of Clomiphene Pills Prescribed

Variable	Cases of breast cancer	Controls	Crude RR (95% CI)	Adjusted RR[a] (95 % CI)	Adjusted *p*-value
Number of Pills					
1-15	54	398	1.36 (1.19-1.55)	1.22 (1.07-1.39)	0.0027
16-30	63	462	1.37 (1.21-1.54)	1.27(1.13-1.44)	<0.0001
31-45	26	134	1.94 (1.58-2.40)	1.71 (1.39-2.11)	<0.0001
46-60	22	175	1.26 (1.03-1.54)	1.13 (0.93-1.38)	0.2214
61+	46	335	1.38 (1.20-1.58)	1.22 (1.06-1.41)	0.0052

[a] Adjusted for BMI, smoking, alcohol use, parity, hormonal contraceptive use, HRT use and oophorectomy

Table 4.4: Association Between Clomiphene and Breast Cancer – Stratified by Diagnoses of PCOS

Variable		Cases of breast cancer	Controls	Crude RR (95% CI)	Adjusted RR[a] (95% CI)	Adjusted *p*-value
PCOS status	**Clomiphene**					
No (n = 295,022)	Exposed	180	1,334	1.36 (1.26-1.45)	1.24 (1.16-1.33)	<0.0001
	Unexposed	26,576	266,932			
Yes (n = 2,264)	**Clomiphene**					
	Exposed	31	172	1.37 (1.13-1.67)	1.22 (0.99-1.50)	0.0605
	Unexposed	239	1,822			

[a] Adjusted for BMI, smoking, alcohol use, parity, hormonal contraceptive use, HRT use and oophorectomy

Chapter 5: General Discussion, Conclusions and Frameworks for Future Research

5.1: General Discussion

The results of the case-control study were unexpected in the context of the existing literature on this subject. As discussed in the previous section, the possibility that the case-control study's results were confounded by the underlying effects of infertility is significant. Its results, therefore, should be interpreted with caution. Nevertheless, it is important to explore lines of evidence which support the postulated association between ovarian stimulation and breast cancer.

A study by Lundberg et al. compiled a cohort of 43,313 women 40 to 69 years old who were enrolled in a mammographic screening program (Lundberg et al., 2016). All members of the study population were never diagnosed with a previous breast cancer nor were they undergoing further testing for a suspected breast malignancy. Subjects completed questionnaires assessing for a history of infertility and a history of ovarian-stimulating fertility treatments. Those with a history of infertility were stratified into three categories based on the type of previous fertility treatment they received: ovarian stimulation with clomiphene or low-dosage gonadotropin cycling, ovarian stimulation with high-dose gonadotropins for IVF, and no previous ovarian stimulation. Breast density was quantitatively assessed by a software program which computed measures of absolute breast density by integrating the percent density of each pixel of the mammogram over total breast volume (Garcia et al., 2017). There were 1,576 women with a history high-dose gonadotropin stimulation for IVF. On average, this group had 3.12 cm^3, 95% CI [2.22-4.02] higher absolute breast density volume compared to women with a history of infertility with no previous treatment. Those with a history of ovarian stimulation with

clomiphene or low-dose gonadotropins did not have a significantly higher than average breast density when compared to the same reference group. The statistical model was adjusted for important confounders such as age at menarche, HRT use, BMI, smoking history, alcohol use, and parity.

Sprague et al. conducted a similar study utilizing a cohort of 1,009 subjects undergoing mammography screening regimens with no history of breast cancer (Sprague et al., 2008). Subjects were assessed for exposure to either clomiphene or gonadotropins through pharmacy records. No stratification based on type of drug used, or drug dosage, was performed. Breast density was qualitatively evaluated by board-certified radiology specialists. Mammograms were classified as being of either low or high density. Overall, this study found no association between ovarian-stimulating therapies and increased breast density, OR 1.06, 95% CI [0.63-1.77], however, the authors reported an increasing trend between breast density and time since exposure. Each additional month following drug dispensal was associated with a 13%, 95% CI [1%-27%] increase in the risk of a subject being classified as having dense breast tissue. Subjects whose mammogram was performed more than a year following drug dispensal were significantly more likely to be classified as having high breast density than those whose mammogram occurred within a year of drug dispensal. Although the study found no overall association, this could be due to the short follow-up time of the study. Only exposures occurring 2 years prior to mammography were considered. Additionally, exposures were assessed through pharmacy records. Therefore, the date of drug dispensal would not correspond with the date of actual exposure. A lag period, of undetermined length, existed between drug dispensal and drug administration. The authors of this study suggested that if the follow-up period was lengthened, the overall association may have approached or entered significance.

Increased breast density is a validated risk factor for breast cancer. A multi-centre case-control study published in the New England Journal of Medicine reported that the risk of breast cancer in women with dense breast tissue is significantly elevated for up to 8 years following initial mammography (Boyd et al., 2007). The National Cancer Institute presently classifies dense breast tissue as an independent risk factor for breast cancer (National Cancer Institute, 2018). Breast density is representative of the composition of mammary tissue. Breast tissue of greater density is associated with greater epithelial cell proliferation, greater collagen levels, and greater volume of glandular structures, which includes the lactiferous ducts and lobules. In simpler terms, increased breast density is representative of an increased volume of the breast composed of epithelial and stromal tissue (Clemons & Goss, 2001). These findings are compatible with the differing radiography characteristics of tissues composing the mammary gland. On mammography, adipose tissue is relatively translucent whereas glandular and connective tissue appear to be of increased radiographic density (Lu et al., 2012). As presented in Chapter 2, estrogen possesses well-characterized effects on glandular proliferation. Breast density has been shown to be related with hormonal factors such age of first birth, total parity, and HRT use (Clemons & Goss, 2001; El-Bastawissi, White, Mandelson, & Taplin, 2000; Hou et al., 2013). As a result, it may be postulated that the supraphysiologic estrogen levels seen during ovarian stimulation contribute to increased breast density through its effects on epithelial proliferation and glandular expansion.

Another result from the case-control study worthy of further discussion is the finding that clomiphene use was only associated with pre-menopausal breast cancers, whereas IVF was associated with both pre and post-menopausal malignancies. For the sake of simplicity, pre-menopausal malignancies, that is, those diagnosed between 18 to 49 years of age, will be referred

to as early-onset (EO). Post-menopausal malignancies, or those diagnosed after 50 years of age, will be referred to as late-onset (LO).

Breast cancer incidence rates in the general population follow a bimodal pattern. Incidence rates peak both at the perimenopausal period and at 70 years of age, "representing the central tendencies for early-onset and late-onset breast cancers (Anderson, Rosenberg, Prat, Perou, & Sherman, 2014)." The majority of EO and LO breast cancers are distinct in their incidence rates and tumor characteristics. According to the Surveillance, Epidemiology and End Results' Cancer Statistics Review, a quarter (25.3%) of breast cancers diagnosed in the United States are EO, with the remaining three quarters being LO (Howlader N, 2017). EO breast cancers are typically more invasive, have a faster growth rate, and are more likely to be resistant to hormonal therapies such as tamoxifen as they are less likely to express the estrogen receptor than their LO counterparts (Anders, Johnson, Litton, Phillips, & Bleyer, 2009; Benz, 2008).

The distinct differences between EO and LO breast malignancies suggest that they have differing etiologies. The initiation stages of EO tumours occur earlier in a woman's life, and they are strongly related to hereditary genetic factors. For example, women with at least one first-degree relative with breast cancer are also at an increased risk of developing EO breast cancer (Lynch, Watson, Conway, Fitzsimmons, & Lynch, 1988). The average age of diagnosis in women with a BRCA1 mutation is also relatively young, with estimates ranging from 40-45 years of age (Brose et al., 2002; Eerola et al., 2005). Conversely, the initial transformation stages of LO tumours occur later in life, during the mid-reproductive years (Benz, 2008).

The relationship between hormonal factors and age of breast cancer diagnosis is not well characterized. As discussed previously, estrogen-receptor signalling may act to promote both the initiation and progression phases of breast tumorigenesis. Therefore, an increase in the exposure of breast tissue to estrogen can theoretically play a role in both EO and LO breast cancer

development. This would occur as the hormone can promote the progression of EO tumours or promote the initiation and progression of LO tumours.

When studied observationally, the association between hormonal factors and age of breast cancer diagnosis is less clear. Certain hormonal factors such as age of menarche, age of first birth, and parity are associated with both EO and LO cancers (Dartois et al., 2016). Conversely, increased BMI has been shown to be more strongly associated with LO malignancies (Lauby-Secretan et al., 2016). Combined oral contraceptive use is associated with only a transient increase in breast cancer risk during their use, and for up to ten years following the cessation of use (Collaborative Group on Hormonal Factors in Breast, 1996). For women using HRT, breast cancer risk is transiently increased during its use and for a 5-year period following cessation of use. (Collaborative Group on Hormonal Factors in Breast Cancer, 1997).

The nebulous relationship between hormonal factors and the age of breast cancer diagnosis makes the interpretation of our results difficult. A possible explanation may lie in three main concepts: ovarian stimulation is only offered to patients of reproductive ages, serum estrogen levels are increased only transiently during treatment, and serum estrogen levels during ovarian stimulation for IVF are significantly higher than estrogen concentrations during ovarian stimulation using clomiphene. When a woman of reproductive age undergoes ovarian stimulation, her risk of breast cancer may be transiently increased as the increased serum estrogen levels may promote the progression of pre-existing breast lesions. This phenomenon would be similar to the transient increase in breast cancer risk seen with oral contraceptives use and HRT use (Collaborative Group on Hormonal Factors in Breast, 1996; Collaborative Group on Hormonal Factors in Breast Cancer, 1997). As ovarian stimulation is only offered to women of reproductive ages, a transient increase in breast cancer risk would lead to an increase in the incidence of EO breast malignancies. An explanation as to why IVF is associated with LO breast

cancers whereas clomiphene is not may be due to the difference in peak estrogen levels seen between the two treatments. As discussed in Chapter 2, ovarian stimulation for IVF results in serum estrogen concentrations which are 2-3 times higher than the levels seen during ovarian stimulation using clomiphene. In a purely speculative capacity, it is possible that the estrogen levels seen during IVF are high enough to have a genotoxic effect on breast tissue which would fundamentally alter its composition. To recall, Lundberg et al.'s study found prior ovarian stimulation for IVF was significantly associated with increased breast density whereas stimulation protocols using clomiphene or low-dose gonadotropins were not (Lundberg et al., 2016). The estrogen levels caused by IVF may act as an initiator of breast tumorigenesis which would alter breast cancer risk over a longer period of time.

5.2: Conclusion

The safety of ovarian-stimulating fertility treatments is an important clinical question. This association was explored in depth by discussing its biological plausibility, overviewing the current observational evidence on the topic, and conducting a novel study which aimed to improve upon many of the limitations affecting previous analyses. For example, our study used longitudinal, validated, prospectively-collected information to evaluate exposure, outcome, and covariate variables. The study also had high statistical power owing to its large study population. Nevertheless, the possibility that its results were confounded by the underlying effects of infertility in addition to the possibility that surveillance bias skewed results necessitates the need to interpret our study with caution. Furthermore, the current literature on this topic generally refutes the notion that these fertility treatments are associated with an increased risk of breast cancer. While this relationship has a strong biological plausibility, in addition to certain lines of evidence linking ovarian stimulation to an increased breast density, there is no definitive

observational evidence of a causal link between ovarian-stimulating fertility treatments and breast cancer. Nevertheless, it is imperative that the safety of these fertility treatments continues to be monitored as they are becoming increasingly prevalent. Additionally, it should be emphasized that while, presently, there is no definitive link between ovarian stimulation and breast cancer, the population of women suffering from fertility-related issues may be inherently at an increased risk of developing breast cancer. Health care professionals should be aware of this phenomenon and offer appropriate guidance and monitoring to this group of patients.

5.3: Framework for Future Research

Monitoring the relationship between ovarian-stimulating fertility treatments and breast cancer is challenging. Firstly, there are several important variables which may confound this relationship of interest. Women affected by infertility are at an inherently higher risk of developing breast cancer as they are more likely to be of lower parity and have a later age of first birth. Additionally, infertility is commonly associated with comorbid conditions and lifestyle factors which predispose a woman to developing breast cancer, such as increased BMI, alcohol us, and a history of tobacco use. Women undergoing treatments such as IVF may also, on average, be of higher socioeconomic status, which is associated with increased health-seeking behaviour. Breast cancer may therefore be preferentially detected in this group of women which contributes to surveillance bias. Secondly, many previous studies have relied on retrospectively collected data to assess exposure. They additionally often performed database linkages. Together, these factors have caused many previous studies to be susceptible to both recall and linkage biases. It is strongly suggested that future research initiatives ensure a robust control of confounding due to the underlying effects of infertility and its associated conditions. Ideally, the study population should be restricted to women affected by infertility. Further adjustment for

socioeconomic status should be strongly considered. The use of prospectively collected data compiled in a single longitudinal database to assess exposure, outcome, and covariate information is also strongly recommended.

Appendix

Search algorithm used to search the EMBED and MEDLINE databases

1. breast cancer.mp.
2. breast carcinoma.mp.
3. breast neoplasm.mp.
4. breast tumor.mp.
5. ductal carcinoma*.mp.
6. lobular carcinoma*.mp.
7. tubullary carcinoma*.mp.
8. medullary carcinoma*.mp.
9. papillary carcinoma*.mp.
10. cribiform carcinoma*.mp.
11. Paget's disease*.mp.
12. mammary tum*.mp.
13. mammary cancer*.mp.
14. **1/or 2/or 3/or 4/or 5/or 6/or 7/or 8/or 9/or 10/or 11/or 12/or 13**
15. ovulation ind*.mp.
16. ovulation stim*.mp.
17. ovulatory stim*.mp.
18. ovarian stim*.mp.
19. controlled ovarian stim*.mp.
20. fertility treat*.mp.
21. infertility treat*.mp.
22. fertility drug* .mp.
23. clomifene.mp.
24. clomifene citrate.mp.
25. clomiph*.mp.
26. fertility promoting agent.mp.
27. fertilization in vitro.mp.
28. IVF*.mp.
29. ICSI*.mp.
30. gonadotropin*.mp.
31. human menopausal gonadotropin.mp.
32. human chorionic gonadotropin.mp.
33. **15/or 16/or 17/or 18/or 19/or 20/or 21/or 22/or 23/or 24/or 25/or 26/or 27/or 28/or 29/or 30/or 31/or 32**
34. 35, restricted to Jan 1, 1995 to Jan 1, 2017, article, full-text available, English language

Exposure Codes

Table A.1: Codes used to identify exposure to clomiphene within the CPRD

Product Code	Product Name
1775	Clomid 50mg tablets (Sanofi)
50064	Clomid 50mg tablets (Necessity Supplies Ltd)
22264	Serophene 50mg tablet (Serono Ltd)
37046	Clomiphene 50mg tablet
19128	Clomifene citrate 50mg tablet (C P Pharmaceuticals)
1271	Clomifene 50mg tablet
18962	Clomifene 50 mg tablets (Wockhardt UK Ltd)
34862	Clomifene citrate 50mg tablet (IVAX Pharmaceuticals)
4415	Clomid 100mg tablet

Table A.2: Codes used to identify exposure to IVF within the CPRD

Medical Code	Read Term
52626	In vitro fertilisation (IVF)
89966	IVF with donor sperm
91910	In vitro fertilisation with donor sperm
64063	IVF with donor eggs
94632	IVF with intracytoplasmic sperm injections (ICSI)
57000	In vitro fertilisation with ICSI
86010	In vitro fertilisation with intra-cytoplasmic sperm injection
97981	IVF intracytoplasmic sperm injection (ICSI) and donor egg
97044	In vitro fertilis intra-cytoplasmic sperm inj and donor egg
90936	IVF NOS
11473	In vitro fertilisation procedure
10238	IVF
1938	In-vitro fertilisation

Outcome Codes

Table A.3: Codes used to identify diagnoses of breast cancer within the CPRD

Medical Code	Read Term
348	Ca female breast
3968	Malignant neoplasm of female breast
20685	Malignant neoplasm of axillary tail of female breast
23380	Malignant neoplasm of nipple of female breast
23399	Malignant neoplasm of upper-outer quadrant of female breast
26853	Malignant neoplasm of nipple and areola of female breast
29826	Malignant neoplasm of upper-inner quadrant of female breast
31546	Malignant neoplasm of central part of female breast
38475	Malignant neoplasm of other site of female breast NOS
42070	Malignant neoplasm of lower-outer quadrant of female breast
45222	Malignant neoplasm of lower-inner quadrant of female breast
56715	Malignant neoplasm of other site of female breast
59831	Malignant neoplasm of nipple or areola of female breast NOS
64686	Malignant neoplasm of areola of female breast
95057	Malignant neoplasm of ectopic site of female breast

Covariate Codes

A.4: BMI

Information on subject weight (in kilograms) and height (in metres) were extracted. BMI was calculated using the formula: BMI = weight/(height^2). The weight and height values inputted closest to the index date were used.

Subjects were categorized into 4 categories (underweight, normal weight, overweight, obese) according to the WHO's standards ("World Health Organization Global Database on Body Mass Index ").

Table A.4: BMI categories

BMI Value	Category
<18.50 kg/m^2	Underweight
18.50-24.99 kg/m^2	Normal Weight
25.00-29.99 kg/m^2	Overweight
≥30.00 kg/m^2	Obese

Table A.5: Codes used to evaluate smoking status within the CPRD

Medical Code	Read Term
33, 60, 11788	Non-smoker
1878, 12944, 12958, 12961	Light-moderate smoker
3568, 1822	Heavy smoker
12956, 12959	Ex-heavy smoker

Table A.6: Codes used to evaluate alcohol use within the CPRD

Medical Code	Read Term
12949, 12970, 4447	Non-drinker
385, 749, 26472, 12980, 12972	Light-moderate drinker
12982, 8999, 1618, 19494, 1399, 12977, 12984	Heavy drinker
12983, 19493	Ex-heavy drinker

Table A.7: Codes used to evaluate parity status within the CPRD

Medical Code	Read Term
167	Normal delivery
239	Spontaneous vaginal delivery
4780	Normal delivery in a completely normal case
13352	Trauma to perineum and vulva during delivery
720	Caesarean delivery
5868	Spontaneous vertex delivery
740	Elective caesarean delivery
6452	Forceps delivery
15480	Born by normal vaginal delivery
17492	FTND - Full term normal delivery
12118	Emergency caesarean section
364	Lower uterine segment caesarean section (LSCS) NEC
8122	Outcome of delivery
1279	Forceps cephalic delivery
618	Delivery by emergency caesarean section
9791	ND - Normal delivery
863	Caesarean section - pregnancy at term
4638	Other caesarean delivery
14686	Forceps cephalic delivery NOS
10049	Elective lower uterine segment caesarean section (LSCS)
6141	Ventouse delivery
688	Ventouse delivery
13315	Born by caesarean section
4182	H/O: delivery no details
463	Breech delivery
2458	Vacuum delivery
13316	Born by emergency caesarean section
6078	H/O: normal delivery
9800	Delivery by elective caesarean section
17744	Lower uterine segment caesarean delivery NEC
8967	Normal delivery NOS

43563	[X]Delivery
6619	H/O: caesarean section
24039	H/O: full term delivery
15361	Born by elective caesarean section
9383	Normal delivery
14727	Born by forceps delivery
3085	Elective caesarean delivery NOS
15675	Born by ventouse delivery
3108	Episiotomy to facilitate delivery
11530	Vaginal delivery
14892	Vacuum delivery NOS
9668	Elective lower uterine segment caesarean delivery
19600	Second degree perineal tear during delivery
25681	SVD - Spontaneous vaginal delivery
12014	[V]Outcome of delivery
58721	Normal delivery in completely normal case NOS
25223	Vacuum extractor delivery
12052	Delivery normal
9160	Spontaneous vertex delivery
11829	H/O: premature delivery
11194	Spontaneous vaginal delivery
20425	Neville - Barnes forceps delivery
11532	Breech extraction delivery
7670	Caesarean delivery NOS
5213	Low forceps cephalic delivery
3365	Premature delivery
19356	Delivery - sex of baby
5522	Complications of labour and delivery NOS
35190	Other caesarean delivery NOS
19599	First degree perineal tear during delivery
11650	Third degree perineal tear during delivery
7916	Delivered by caesarean section - pregnancy at term
15828	Born by breech delivery
5866	Induction and delivery operations
40710	Forceps delivery NOS
30274	Low forceps delivery
15167	Early onset of delivery
17684	Kielland forceps delivery
23394	Mid forceps cephalic delivery NEC
23994	Assisted breech delivery
15256	Unspecified perineal laceration during delivery
2943	Assisted breech delivery
21554	Kielland forceps cephalic delivery with rotation
28726	Water birth delivery
31743	Normal delivery but ante- or post- natal conditions present

7377	[X]Infection of caesarean section wound following delivery
19609	Vaginal tear during delivery
33477	Caesarean delivery - delivered
42275	Mid-cavity forceps delivery
49446	Vacuum extractor delivery - delivered
50675	Forceps delivery - delivered
43959	Outcome of delivery NOS
28100	Caesarean wound disruption
53937	Forceps delivery unspecified
28903	Delivered by low forceps delivery
29885	Complications occurring during labour and delivery
26712	Labial tear during delivery
19605	Second degree perineal tear during delivery NOS
17078	H/O: previous forceps delivery
34411	Delivered by mid-cavity forceps delivery
52875	Caesarean delivery unspecified
19602	Second degree perineal tear during delivery, unspecified
5721	Urinary tract infection following delivery
33773	Other specified normal delivery
22491	Spontaneous breech delivery
4786	Multiple delivery, all by caesarean section
5033	High forceps cephalic delivery NEC
37699	Vacuum extractor delivery NOS
20738	Manually assisted vaginal delivery
33480	Other specified forceps cephalic delivery
25157	Early onset of delivery - delivered
29070	Spontaneous breech delivery
19608	First degree perineal tear during delivery NOS
19603	First degree perineal tear during delivery, unspecified
15926	Other specified other breech delivery
20002	High forceps cephalic delivery with rotation
33378	Other breech delivery
36037	[X]Vaginitis following delivery
17704	Simpson's forceps delivery
32590	Delivery problem
33915	Multiple delivery
29155	Caesarean hysterectomy
56310	Delivery observations
40876	Third degree perineal tear during delivery NOS
31954	Number of caesarean sections
39576	Fourth degree perineal tear during delivery
37054	Second degree perineal tear during delivery - delivered
18287	Normal delivery of placenta
19604	Third degree perineal tear during delivery, unspecified
50293	Induction and delivery operations NOS

43760	Other method of delivery NOS
15514	Upper uterine segment caesarean delivery NEC
47546	Other specified elective caesarean delivery
37878	Multiple birth delivery
40706	Unspecified perineal laceration during delivery NOS
32380	Vulval delivery trauma
39168	Third degree perineal tear during delivery - delivered
40744	Abnormal delivery
15073	Other specified other caesarean delivery
31280	Vulval tear during delivery
83535	[X]Other single spontaneous delivery
29872	History of past delivery
34628	Failed forceps delivery
31254	First degree perineal tear during delivery - delivered
54591	Breech extraction delivery NOS
42324	Vulval/perineal trauma during delivery NOS
55543	Other operations to facilitate delivery
34399	[X]Cervicitis following delivery
44235	Vaginal delivery following previous caesarean section
44494	Caesarean wound disruption NOS
43918	Fourchette tear during delivery
47508	Other methods of delivery
57789	Delivery by combination of forceps and vacuum extractor
36137	Early onset of delivery NOS
36703	Vulval and perineal haematoma during delivery
50847	Caesarean delivery following previous Caesarean delivery
31780	Spontaneous breech delivery
9625	Labour and delivery complicated by fetal heart rate anomaly
64464	Other breech delivery NOS
61259	Elective upper uterine segment caesarean delivery
27731	Vulval and perineal haematoma during delivery
60953	Precipitate delivery
58844	Vacuum extractor delivery unspecified
48230	Deliveries by spontaneous breech delivery
69920	Complications of labour and delivery NOS
61212	Other vulval and perineal trauma during delivery
33884	Multiple delivery, all spontaneous
53572	Other specified complications of labour or delivery
61077	Mid forceps cephalic delivery with rotation
52151	[X]Labour+delivery complications/other evidence of fetal distress
34102	Incomplete placenta at delivery
60538	Multiple delivery, all by forceps and vacuum extractor
19607	Unspecified perineal laceration during delivery, unspecified
28861	Delivery by caesarean hysterectomy
47157	Vulval/perineal trauma during delivery NOS

23866	Symphysiotomy to facilitate delivery
34333	Incomplete delivery of placenta
57652	Fourth degree perineal tear during delivery NOS
59941	Early onset of delivery unspecified
61578	Caesarean wound disruption unspecified
72022	Fourth degree perineal tear during delivery, unspecified
86399	Other specified vacuum delivery
40875	Second degree perineal tear during delivery with p/n prob
44472	Other complications of labour and delivery
48742	Face delivery
47863	[X]Other single delivery by caesarean section
51736	Low vacuum delivery
66074	Other complications of labour and delivery with p/n problem
67052	Unspecified perineal laceration during delivery - delivered
104873	Premature labour and delivery
50236	Other specified induction or delivery operations
62862	Third degree perineal tear during delivery with p/n problem
67333	[V]Unspecified delivery outcome
24243	Labour and delivery complications by meconium in amniotic fluid
49485	Labour+delivery complicated by biochem evidence/fetal stress
104872	Premature labour with premature delivery
62406	Delayed delivery second twin unspecified
62508	[V]Examination immediately after delivery
96089	Rapid rate of delivery
57957	Other specified other method of delivery
61226	[V]Other specified outcome of delivery
72021	Fourth degree perineal tear during delivery - delivered
73048	Other specified breech extraction delivery
27963	Delayed delivery of second twin, triplet etc.
66219	[X]Other infection of genital tract following delivery
69971	Slow rate of delivery
95780	Complete placenta at delivery
37245	Vulval and perineal haematoma during delivery, unspecified
50924	First degree perineal tear during delivery with p/n problem
66315	[X]Other specified assisted single delivery
69780	[X]Other and unspecified forceps delivery
30805	Other vulval/perineal trauma during delivery, unspecified
39573	Umbilical cord not around baby's neck at delivery
55124	Other complications of labour and delivery NOS
62318	Trial of vacuum delivery
65940	Trial of forceps delivery
30806	Other vulval/perineal trauma during delivery NOS
47036	Problem of pelvis for delivery
54623	Caesarean wound disruption with postnatal complication
57945	Vacuum delivery before full dilation of cervix

60256	Brow delivery
60924	No progress with delivery
66501	Complications of labour and delivery NOS
66843	Unspecified perineal laceration during delivery + p/n prob
67489	Vulval/perineal trauma during delivery NOS unspec
72513	Extraperitoneal caesarean section
93273	Complications of labour and delivery NOS - delivered
100675	[X]Assisted single delivery, unspecified
61597	Fourth degree perineal tear during delivery with p/n problem
70242	Barton forceps cephalic delivery with rotation
70615	Vulval and perineal haematoma during delivery NOS
71057	Vulval and perineal haematoma during delivery - delivered
90497	Complications of labour and delivery NOS with p/n problem
99608	Other operation to facilitate delivery NOS
100597	Other complications of labour and delivery - delivered
105032	Premature labour without delivery
22498	Rate of delivery
42572	Delayed delivery second twin - delivered
42885	Observation of pattern of delivery
57488	Vulval/perineal trauma during delivery NOS - delivered
65331	Other vulval/perineal trauma during delivery + p/n problem
67410	[X]Other genitourinary tract infections following delivery
68752	Normal rate of delivery
71862	Delayed delivery second twin with antenatal problem
90885	[X]Labour+delivery complicated by other cord entanglement
95858	Other vulval/perineal trauma during delivery- delivered
98059	[X]Other manipulation-assisted delivery
98749	Incision of cervix to facilitate delivery
99361	Vulval and perineal haematoma during delivery + p/n problem
101229	Observation of delivery push in labour
103319	[X]Complications of labour and delivery
105276	Breech extraction delivery with version
105419	High vacuum delivery
106001	Destructive operation for delivery
106113	[X]Multiple delivery, unspecified
106808	Vulval/perineal trauma during delivery NOS with p/n problem
40729	Gravida 1
54604	Gravida 2
25612	Gravida 3
54572	Gravida 4
54597	Gravida 5
58003	Gravida 6
61894	Gravida 7
46527	Primigravida
86753	Gravida 8

94244	Gravida 9
63196	Multigravida
8776	[X] Stillbirth
23330	GP unit birth
1825	Twin birth
6184	[V]Normal pregnancy
5709	Pregnancy care
29662	Multiparous

Table A.8: Codes used to evaluate hormonal contraceptive use within the CPRD

Product Codes	Product Term
65068	Yacella 0.03mg/3mg tablets (Morningside Healthcare Ltd)
65312	Eloine 0.02mg/3mg tablets (Bayer Plc)
68515	Teragezza 2000microgram/35microgram tablets (Morningside Healthcare
65475	Generic Femodene ED tablets
37073	Sunya 20/75 tablets (Stragen UK Ltd)
44229	Millinette 20microgram/75microgram tablets (Consilient Health Ltd
2265	Etynodiol 500microgram tablets
61088	Generic Logynon tablets
8176	Ethinylestradiol 20microgram / Norethisterone acetate 1mg tablets
3436	Ortho-novin 1/50 Tablet (Janssen-Cilag Ltd)
15987	Ethinylestradiol with norethisterone - triphasic 7 x 35+500mcg; 7 x
18569	Gestodene with ethinylestradiol 75microgramwith20microgram Tablet
65786	Ethinylestradiol 30microgram / Desogestrel 150microgram tablets (AM
14670	Ethinylestradiol with norethisterone - biphasic 7 x 35mcg+500mcg; 14 x
63725	Dretine 0.03mg/3mg tablets (Teva UK Ltd)
13209	Etonogestrel 68mg implant
81w03	Conova 30 Tablet (Pharmacia Ltd)
54527	Cerelle 75microgram tablets (Consilient Health Ltd)
45059	Ethinylestradiol with levonorgestrel 30micrograms + 50micrograms Tablet
2061	Norethisterone 350microgram tablets
3693	Triadene tablets (Bayer Plc)
36829	Katya 30/75 tablets (Stragen UK Ltd)
19131	Ethinylestradiol with gestodene 30micrograms+75micrograms Tablet
57181	Lizinna 250microgram/35microgram tablets (Morningside Healthcare Ltd)
23211	Desogestrel with ethinylestradiol 150micrograms with 30micrograms tablets
239	Micronor 350microgram tablets (Janssen-Cilag Ltd)
47132	Co-cyprindiol 2mg+35microgram Tablet (Sandoz Ltd)
1720	Levonorgestrel 30microgram tablets
49214	Cilest 35microgram/250microgram tablets (Mawdsley-Brooks & Company
40650	Ethinylestradiol with norethisterone 35micrograms + 750micrograms Tablet

61190	Co-cyprindiol 2000microgram/35microgram tablets (A A H Pharmaceuticals
41	Microgynon 30 tablets (Bayer Plc)
16624	Levonorgestrel 228mg Implant
44046	Rigevidon tablets (Consilient Health Ltd)
14459	Gynovlar 21 Tablet
11910	Ethinylestradiol 20microgram / Gestodene 75microgram tablets
5862	Norinyl-1 tablets (Pfizer Ltd) Norethisterone/Mestranol
14601	Ethinylestradiol 35microgram / Norgestimate 250microgram tablets
68352	Aidulan 30microgram/75microgram tablets (Lupin (Europe) Ltd)
4964	Femodette tablets (Bayer Plc) Gestodene/Ethinylestradiol
25124	Acnocin 2000microgram/35microgram tablets (Sandoz Ltd)
1601	Trinovum tablets
29499	Ethinylestradiol 33.9micrograms/24hours / Norelgestromin
4608	Dianette tablets (Mylan Ltd) Cyproterone acetate/Ethinylestradiol 2mg + 35microgram
2856	Norimin 1mg/35microgram tablets (Pfizer Ltd)
8482	Ethinylestradiol 35microgram / Norethisterone 1mg tablets
59503	Lucette 0.03mg/3mg tablets (Consilient Health Ltd)
44278	TriRegol tablets (Consilient Health Ltd)
936	Femodene tablets (Bayer Plc)
1613	Microval 30microgram tablets (Wyeth Pharmaceuticals) Levonorgestrel
56483	Generic Tri-Minulet tablets
56103	Cerelle 75microgram tablets (Consilient Health Ltd)
62968	Maexeni 150microgram/30microgram tablets (Lupin (Europe) Ltd)
44336	Gedarel 30microgram/150microgram tablets (Consilient Health Ltd)
443	Desogestrel with ethinylestradiol 150micrograms with 20micrograms tablets
977	Minulet tablets (Wyeth Pharmaceuticals)
6431	Co-cyprindiol 2000microgram/35microgram tablets
47057	Yasminelle 3mg+20microgram Tablet (Bayer Plc)
31528	Ethinylestradiol with norethisterone - triphasic 7x35+500mcg;
10201	Desogestrel 75microgram tablets
53201	Dianette tablets (Lexon (UK) Ltd)
44994	Millinette 30microgram/75microgram tablets (Consilient Health Ltd)
3472	Trinovum ed ED tablets (Janssen-Cilag Ltd)
9592	Implanon 68mg implant (Organon Laboratories Ltd)
64918	Aidulan 20microgram/75microgram tablets (Lupin (Europe) Ltd)
56732	Aizea 75microgram tablets (Besins Healthcare (UK) Ltd)
13248	Ethinylestradiol 30microgram / Desogestrel 150microgram tablets
7814	Ethinylestradiol 35microgram / Norethisterone 500microgram tablets
43003	Levest 150/30 tablets (Morningside Healthcare Ltd)
14977	Ethinylestradiol with gestodene - triphasic 6 x 30+50mcg; 5 x 40+70mcg; 10
1062	Ovranette 150microgram/30microgram tablets (Pfizer Ltd)
1378	Mercilon 150microgram/20microgram tablets (Merck Sharp & Dohme Ltd)
12631	Ethinylestradiol with levonorgestrel and placebo 30micrograms +
6596	Norelgestromin with ethinylestradiol 203micrograms +

61890	Desogestrel 75microgram tablets (A A H Pharmaceuticals Ltd)
1352	Loestrin 30 tablets (Galen Ltd)
25263	Norgestimate with ethinylestradiol 250micrograms + 35micrograms Tablet
18823	Ethinylestradiol 30microgram / Norethisterone acetate 1.5mg tablets
5986	Cerazette 75microgram tablets (Merck Sharp & Dohme Ltd)
1354	Brevinor 500microgram/35microgram tablets (Pfizer Ltd)
15886	Ethinylestradiol with levonorgestrel - triphasic 6x30+50mcg; 5x40+75mcg;
52443	Microgynon 30 tablets (Mawdsley-Brooks & Company Ltd)
44196	Nexplanon 68mg implant (Merck Sharp & Dohme Ltd)
19551	Controvlar Tablet (Schering Health Care Ltd)
697	Yasmin tablets (Bayer Plc)
38500	Co-cyprindiol 2000microgram/35microgram tablets (Fannin UK Ltd)
7776	Ethinylestradiol 30microgram / Gestodene 75microgram tablets
6686	Ethinylestradiol 30microgram / Levonorgestrel 150microgram tablets
58642	Cimizt 30microgram/150microgram tablets
55819	Nacrez 75microgram tablets (Teva UK Ltd)
45557	Levest 150/30 tablets (Actavis UK Ltd)
2026	Logynon ED tablets (Bayer Plc)
42510	Ethinylestradiol with levonorgestrel Tablet
57264	Estradiol 1.5mg / Nomegestrol 2.5mg tablets
60739	Yaz tablets (Imported (United States))
44457	Gedarel 20microgram/150microgram tablets (Consilient Health Ltd)
59414	Desogestrel 75microgram tablets (Actavis UK Ltd)
47281	Elevin 150microgram/30microgram tablets (MedRx Licences Ltd)
2769	Cyproterone acetate with ethinylestradiol 2mg with 35micrograms tablets
6166	Evra transdermal patches (Janssen-Cilag Ltd)
67248	Dianette tablets (Waymade Healthcare Plc)
65711	Daylette 0.02mg/3mg tablets (Consilient Health Ltd)
2084	Ovran 30 Tablet (Wyeth Pharmaceuticals)
68841	Ethinylestradiol 30microgram / Drospirenone 3mg tablets (Colorama
2553	Norgeston 30microgram tablets (Bayer Plc)
66154	Cilique 250microgram/35microgram tablets (Consilient Health Ltd)
1466	Femulen 500microgram tablets (Pfizer Ltd)
935	Marvelon tablets (Merck Sharp & Dohme Ltd)
67318	Evra transdermal patches (Dowelhurst Ltd)
40618	Estradiol valerate (estradiol valerate with dienogest) tablets
63258	Munalea 150microgram/30microgram tablets (Lupin (Europe) Ltd)
21343	Minovlar ed Tablet (Schering Health Care Ltd)
2354	Ovysmen 500microgram/35microgram tablets (Janssen-Cilag Ltd)
1988	Binovum tablets (Janssen-Cilag Ltd)
125	Dianette tablets (Bayer Plc) Cyproterone acetate/Ethinylestradiol 2mg +
21733	Gestodene with ethinylestradiol 75microgramwith30microgram Tablet
23218	Ethinylestradiol with cyproterone acetate 35microgram with 2mg tablets
2819	Norplant 228mg Implant (Hoechst Marion Roussel)
61465	Feanolla 75microgram tablets (Lupin (Europe) Ltd)

56539	Zoely 2.5mg/1.5mg tablets (Merck Sharp & Dohme Ltd)
67274	Dianette tablets (Sigma Pharmaceuticals Plc) Cyproterone
5576	Synphase tablets (Pfizer Ltd)
16110	Ethinylestradiol 20microgram / Desogestrel 150microgram tablets
6716	Ethinylestradiol 30microgram / Drospirenone 3mg tablets
3471	Femodene ED tablets (Bayer Plc)
58485	Zelleta 75microgram tablets (Morningside Healthcare Ltd)
17756	Mestranol 50microgram / Norethisterone 1mg tablets
4917	Microgynon 30 ED tablets (Bayer Plc)
9119	Minilyn Tablet (Organon Laboratories Ltd)
1427	Loestrin 20 tablets (Galen Ltd)
65005	Ethinylestradiol 20microgram / Drospirenone 3mg tablets
1071	Cilest 35microgram/250microgram tablets (Janssen-Cilag Ltd)
978	Logynon tablets (Bayer Plc)

Table A.9: Codes used to evaluate usage of hormone replacement therapy within the CPRD

Product code	Product name
206	Estraderm TTS 100 patches (Novartis Pharmaceuticals UK Ltd)
986	Estraderm TTS 50 patches (Novartis Pharmaceuticals UK Ltd)
988	Estraderm TTS 25 patches (Novartis Pharmaceuticals UK Ltd)
1488	Evorel -50 50microgram/24hr (3.2mg/unit) Transdermal patch (Janssen-Cilag
1489	Evorel -75 75microgram/24 hr(4.8mg/unit) Transdermal patch (Janssen-Cilag
1797	Oestrogel 0.06% Gel (Aventis Pharma)
2140	FemSeven 50 patches (Teva UK Ltd)
2141	Evorel -25 25microgram/24hr (1.6mg/unit) Transdermal patch (Janssen-Cilag
2397	Evorel -100 100microgram/24 hr (6.4mg/unit) Transdermal patch (Janssen-
2977	Estradiol 50mg implant
3040	Estraderm MX 50 patches (Novartis Pharmaceuticals UK Ltd)
3387	Estraderm MX 25 patches (Novartis Pharmaceuticals UK Ltd)
3433	Sandrena 0.10% Gel (Organon Laboratories Ltd)
4328	Elleste Solo 1mg tablets (Meda Pharmaceuticals Ltd)
4466	Progynova TS 50microgram/24hr (3.8mg/unit) Transdermal patch (Schering
4467	Progynova TS 100microgram/24 hr (7.6mg/unit) Transdermal patch
4511	Estradiol 100mg implant
4721	Estradiol 75micrograms/24hr twice weekly patch
4882	Estraderm MX 100 patches (Novartis Pharmaceuticals UK Ltd)
4909	Elleste Solo 2mg tablets (Meda Pharmaceuticals Ltd)
4956	Estradiol 1mg tablets
4977	Estraderm MX 75 patches (Novartis Pharmaceuticals UK Ltd)
5005	Estradiol 50micrograms/24hr twice weekly patch
5100	Estradiol 25micrograms/24hr twice weekly patch
5292	Fematrix 40 patches (Abbott Healthcare Products Ltd)

5343	Estradiol 25micrograms/24hr twice weekly patch
5755	Elleste Solo MX 40 transdermal patches (Meda Pharmaceuticals Ltd)
5759	Estradiol 100micrograms/24hr twice weekly patch
6041	Estradiol 0.06% gel
6059	Estradiol 100micrograms/24hr twice weekly patch
6082	FemSeven 75 patches (Teva UK Ltd)
6177	FemSeven 100 patches (Teva UK Ltd)
6563	Estradiol 75micrograms/24hr once weekly patch
6601	Estradiol 25micrograms/24hr twice weekly patch
6622	Zumenon 2mg tablets (Abbott Healthcare Products Ltd)
6793	Estradiol 40micrograms/24hours transdermal patches
7242	Estradiol 0.1% gel
7381	Estradiol 2mg tablets
7388	Zumenon 1mg tablets (Abbott Healthcare Products Ltd)
8837	Estradiol 25mg implant
9047	Fematrix 80 patches (Abbott Healthcare Products Ltd)
9268	Estradiol 50micrograms/24hr twice weekly patch
9649	Elleste Solo MX 80 transdermal patches (Meda Pharmaceuticals Ltd)
9901	Progynova TS 100micrograms/24hours transdermal patches (Bayer Plc)
10052	Progynova TS 50micrograms/24hours transdermal patches (Bayer Plc)
10076	Estradot 75micrograms/24hours patches (Novartis Pharmaceuticals UK Ltd)
10096	Estradot 100micrograms/24hours patches (Novartis Pharmaceuticals UK Ltd)
10126	Estradot 25micrograms/24hours patches (Novartis Pharmaceuticals UK Ltd)
10180	Estradot 50micrograms/24hours patches (Novartis Pharmaceuticals UK Ltd)
10946	Menorest 50 patches (Novartis Pharmaceuticals UK Ltd)
10967	Menorest 37.5 patches (Novartis Pharmaceuticals UK Ltd)
11375	Estradiol 50micrograms/24hr once weekly patch
11430	Estradiol 25micrograms/24hr once weekly patch
11672	Estradiol 25micrograms/24hr twice weekly patch
11882	Estradiol 50micrograms/24hr twice weekly patch
12773	Estradiol 100micrograms/24hr twice weekly patch
13582	Menorest 75 patches (Novartis Pharmaceuticals UK Ltd)
14234	Estradiol 37.5micrograms/24hours transdermal patches
14580	Dermestril 25 patches (ProStrakan Ltd)
14792	Estradiol 50micrograms/24hr twice weekly patch
15100	Estradiol 37.5micrograms/24hr twice weekly patch
15194	Dermestril 100 patches (ProStrakan Ltd)
15298	Estradiol 25micrograms/24hr twice weekly patch
15328	Estradiol 25micrograms/24hr twice weekly patch
15869	Dermestril 50 patches (ProStrakan Ltd)
15880	Dermestril - Septem 75 patches (ProStrakan Ltd)
16437	Estradiol 100micrograms/24hr once weekly patch
18009	Estradiol 50micrograms/24hr once weekly patch
18218	Estradiol 100micrograms/24hr twice weekly patch
18383	Estradiol 80micrograms/24hours transdermal patches

18437	Estradiol 75micrograms/24hr twice weekly patch
18502	Estradiol 75micrograms/24hr twice weekly patch
18600	Estradiol 75micrograms/24hr twice weekly patch
18901	Estradiol 50micrograms/24hr twice weekly patch
19135	Estradiol 50mg implant (Merck Sharp & Dohme Ltd)
19145	Estradiol 50micrograms/24hr twice weekly patch
19487	Estradot 37.5micrograms/24hours patches (Novartis Pharmaceuticals UK
20135	Estradiol 75micrograms/24hr twice weekly patch
20155	Estradiol 50micrograms/24hr once weekly patch
20888	Adgyn Estro 2mg tablets (ProStrakan Ltd)
20895	Dermestril - Septem 50 patches (ProStrakan Ltd)
21757	Estradiol 100mg implant (Organon Laboratories Ltd)
22335	Estradiol 100micrograms/24hr once weekly patch
26049	Estradiol 100micrograms/24hr twice weekly patch
32930	Dermestril septem 25microgram/24hr Transdermal patch (Strakan Ltd)
35091	Oestrogel Pump-Pack 0.06% gel (Marlborough Pharmaceuticals Ltd)
35742	Estradiol 100micrograms/24hr once weekly patch
35958	Bedol 2mg tablets (ReSource Medical UK Ltd)
37033	Estradiol 75micrograms/24hours transdermal patches
37037	Estradiol 100micrograms/24hours transdermal patches
37692	Estradiol 25micrograms/24hours transdermal patches
37697	Estradiol 50micrograms/24hours transdermal patches
38932	Evorel 50 patches (Janssen-Cilag Ltd)
38935	Evorel 25 patches (Janssen-Cilag Ltd)
38940	Evorel 75 patches (Janssen-Cilag Ltd)
38965	Evorel 100 patches (Janssen-Cilag Ltd)
42515	Estradiol 40micrograms/24 hourspatch
49694	Estraderm TTS 25 patches (Doncaster Pharmaceuticals Ltd)
51201	Estradot 50micrograms/24hours patches (Necessity Supplies Ltd)
51978	Estraderm MX 25 patches (Stephar (U.K.) Ltd)
52174	Estradot 50micrograms/24hours patches (Sigma Pharmaceuticals Plc)
52480	Estraderm MX 25 patches (Lexon (UK) Ltd)
52813	Nuvelle TS Phase I patches (Bayer Plc)
54425	Oestrogel Pump-Pack 0.06% gel (Doncaster Pharmaceuticals Ltd)
56468	Oestrogel Pump-Pack 0.06% gel (Waymade Healthcare Plc)
8465	Ovestin 1mg tablets (Organon Laboratories Ltd)
10593	Estriol 1mg tablets
18311	Estriol 250micrograms tablets
774	Premarin 1.25mg tablets (Pfizer Ltd)
1331	Premarin 0.625mg tablets (Pfizer Ltd)
2433	Premarin 2.5mg tablets (Wyeth Pharmaceuticals)
35198	Premarin 0.3mg tablets (Pfizer Ltd)
3423	Conjugated oestrogens 625microgram tablets
3960	Conjugated oestrogens 1.25mg tablets
35718	Conjugated oestrogens 300microgram tablets

12138	Conjugated oestrogens 2.5mg tablets
37004	Progesterone micronised 100mg capsules
36623	Progesterone micronised 200mg capsules
5569	Pro-gest Cream (Higher Nature Ltd)
33162	Phyto progesterone 1.5% cream
33501	Phyto progesterone cream
41349	Phyto progesterone 3% cream
6235	Pro-Juven cream (Imported (United States))
15984	Pro-Juven 1.5% cream (Imported (United States))
21079	Gestone 10mg/ml Injection (Ferring Pharmaceuticals Ltd)
10761	Progesterone 25mg/1ml solution for injection ampoules
11167	Gestone 25mg/1ml solution for injection ampoules (Ferring Pharmaceuticals
35502	Progesterone 50mg/1ml solution for injection ampoules
35547	Gestone 50mg/1ml solution for injection ampoules (Nordic Pharma Ltd)
36056	Progesterone 100mg/2ml solution for injection ampoules
37004	Progesterone micronised 100mg capsules
3982	Medroxyprogesterone acetate contraceptive 150mg/ml Injection
19391	Depo-provera 500mg (150mg/ml) Injection (Pharmacia Ltd)
3892	Depo-provera 50mg/ml Injection (Pharmacia Ltd)
13811	Medroxyprogesterone acetate 500mg (150mg/ml) Injection
24432	Depo-provera oncology 500mg (150mg/ml) Injection (Pharmacia Ltd)
3445	Medroxyprogesterone acetate contraceptive 50mg/ml Injection
24839	Provera 80mg/ml Liquid (Pharmacia Ltd)
13623	Medroxyprogesterone acetate 80mg/ml Oral suspension
967	Depo-Provera 150mg/1ml suspension for injection vials (Pfizer Ltd)
35001	Depo-Provera 150mg/1ml suspension for injection pre-filled syringes (Pfizer
35075	Medroxyprogesterone 150mg/1ml suspension for injection pre-filled syringes
18059	Medroxyprogesterone 500mg/2.5ml suspension for injection vials
27815	Farlutal 500 suspension for injection 2.5ml vials (Pfizer Ltd)
49518	Depo-Provera 150mg/1ml suspension for injection pre-filled syringes
49666	Depo-Provera 150mg/1ml suspension for injection pre-filled syringes
51351	Depo-Provera 150mg/1ml suspension for injection pre-filled syringes
57037	Sayana Press 104mg/0.65 ml suspension for injection pre-filled disposable
3174	Medroxyprogesterone 10mg tablets
2420	Provera 10mg tablets (Pfizer Ltd)
2421	Medroxyprogesterone 5mg tablets
1266	Provera 5mg tablets (Pfizer Ltd)
2549	Medroxyprogesterone 100mg tablets
3884	Provera 100mg tablets (Pfizer Ltd)
2882	Medroxyprogesterone 200mg tablets
9051	Provera 200mg tablets (Pfizer Ltd)
8750	Provera 400mg tablets (Pfizer Ltd)
11214	Medroxyprogesterone 2.5mg tablets
9058	Provera 2.5mg tablets (Pfizer Ltd)
8761	Medroxyprogesterone 400mg tablets

12230	Medroxyprogesterone 500mg tablets
11439	Farlutal 100 tablets (Pfizer Ltd)
17293	Improvera 1.5mg+10mg Tablet (Pharmacia Ltd)
9078	Farlutal 500 tablets (Pfizer Ltd)
36409	Climanor 5mg tablets (ReSource Medical UK Ltd)
15544	Medroxyprogesterone 250mg tablets
23783	Farlutal 250 tablets (Pfizer Ltd)
30671	Adgyn Medro 5mg tablets (ProStrakan Ltd)
49841	Provera 10mg tablets (Waymade Healthcare Plc)
32248	Piperazine oestrone sulphate 1.5mg with medroxyprogesterone 10mg tablet
339	Premique 0.625mg/5mg tablets (Pfizer Ltd)
6492	Premique Low Dose 0.3mg/1.5mg modified-release tablets (Pfizer Ltd)
6861	Indivina 1mg/5mg tablets (Orion Pharma (UK) Ltd)
7175	Indivina 1mg/2.5mg tablets (Orion Pharma (UK) Ltd)
9671	Indivina 2mg/5mg tablets (Orion Pharma (UK) Ltd)
11859	Conjugated oestrogens 625microgram / Medroxyprogesterone 5mg tablets
11934	Conjugated oestrogens 300microgram / Medroxyprogesterone 1.5mg
16367	Estradiol valerate 1mg / Medroxyprogesterone 2.5mg tablets
16392	Estradiol valerate 2mg / Medroxyprogesterone 5mg tablets
19432	Estradiol valerate 1mg / Medroxyprogesterone 5mg tablets
52101	Premique 0.625mg/5mg tablets (Lexon (UK) Ltd)
34174	Conjugated oestrogens equine with medroxyprogesterone acetate
19429	Conjugat oestrogen equi and (conjugat oestrogen equi with
9235	Norgestrel and conjugated oestrogens (equine) 150micrograms + 1.25mg
9224	Norgestrel and conjugated oestrogens (equine) 150micrograms +
22741	Conjugated oestrogens 625microgram tablets and Norgestrel 150microgram
12970	Conjugated oestrogens 1.25mg tablets and Norgestrel 150microgram tablets
7917	CONJUGATED OESTROGENS 625/NORGESTREL 500 MCG TAB

Table A.10: Codes used to identify oophorectomies within the CPRD

Medical	Read Term
46003	Oophorectomy of remaining solitary ovary
1454	Oophorectomy NEC

Table A11: Codes used to identify diagnoses of polycystic ovary syndrome within the CPRD

Read Code	Read Term
C165.00	Polycystic ovary syndrome
C164.12	Stein-Leventhal syndrome
C164.00	Polycystic ovaries

References

Adashi, E. Y. (1984). Clomiphene citrate: mechanism(s) and site(s) of action--a hypothesis revisited. *Fertil Steril, 42*(3), 331-344.

Adashi, E. Y., Hsueh, A. J., Bambino, T. H., & Yen, S. S. (1981). Disparate effect of clomiphene and tamoxifen on pituitary gonadotropin release in vitro. *Am J Physiol, 240*(2), E125-130. doi:10.1152/ajpendo.1981.240.2.E125

Althuis, M. D., Dozier, J. M., Anderson, W. F., Devesa, S. S., & Brinton, L. A. (2005). Global trends in breast cancer incidence and mortality 1973-1997. *Int J Epidemiol, 34*(2), 405-412. doi:10.1093/ije/dyh414

Amer, S. A., Smith, J., Mahran, A., Fox, P., & Fakis, A. (2017). Double-blind randomized controlled trial of letrozole versus clomiphene citrate in subfertile women with polycystic ovarian syndrome. *Hum Reprod, 32*(8), 1631-1638. doi:10.1093/humrep/dex227

American Society for Reproductive Medicine. (1997). Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril, 67*(5), 817-821.

Amir, E., Freedman, O. C., Seruga, B., & Evans, D. G. (2010). Assessing women at high risk of breast cancer: a review of risk assessment models. *J Natl Cancer Inst, 102*(10), 680-691. doi:10.1093/jnci/djq088

Anders, C. K., Johnson, R., Litton, J., Phillips, M., & Bleyer, A. (2009). Breast cancer before age 40 years. *Semin Oncol, 36*(3), 237-249. doi:10.1053/j.seminoncol.2009.03.001

Anderson, W. F., Rosenberg, P. S., Prat, A., Perou, C. M., & Sherman, M. E. (2014). How many etiological subtypes of breast cancer: two, three, four, or more? *J Natl Cancer Inst, 106*(8). doi:10.1093/jnci/dju165

Azios, N. G., Krishnamoorthy, L., Harris, M., Cubano, L. A., Cammer, M., & Dharmawardhane, S. F. (2007). Estrogen and resveratrol regulate Rac and Cdc42 signaling to the actin cytoskeleton of metastatic breast cancer cells. *Neoplasia, 9*(2), 147-158.

Baerwald, A. R., Adams, G. P., & Pierson, R. A. (2012). Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Hum Reprod Update, 18*(1), 73-91. doi:10.1093/humupd/dmr039

Balakrishnan TR, F. R. (1993). *Infertility among Canadians: an analysis of data from the Canadian Fertility Survey (1984) and General Social Survey (1990)*. Retrieved from Ottawa:

Balmana, J., Diez, O., Castiglione, M., & Group, E. G. W. (2009). BRCA in breast cancer: ESMO clinical recommendations. *Ann Oncol, 20 Suppl 4*, 19-20. doi:10.1093/annonc/mdp116

Barzi, F., & Woodward, M. (2004). Imputations of missing values in practice: results from imputations of serum cholesterol in 28 cohort studies. *Am J Epidemiol, 160*(1), 34-45. doi:10.1093/aje/kwh175

Basen-Engquist, K., & Chang, M. (2011). Obesity and cancer risk: recent review and evidence. *Curr Oncol Rep, 13*(1), 71-76. doi:10.1007/s11912-010-0139-7

Beatson GT. (1896). On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. *Lancet ii*, 104-107.

Benz, C. C. (2008). Impact of aging on the biology of breast cancer. *Crit Rev Oncol Hematol, 66*(1), 65-74. doi:10.1016/j.critrevonc.2007.09.001

Bernstein, L., Hanisch, R., Sullivan-Halley, J., & Ross, R. K. (1995). Treatment with human chorionic gonadotropin and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev, 4*(5), 437-440.

Bhattacharya, S., Porter, M., Amalraj, E., Templeton, A., Hamilton, M., Lee, A. J., & Kurinczuk, J. J. (2009). The epidemiology of infertility in the North East of Scotland. *Hum Reprod, 24*(12), 3096-3107. doi:10.1093/humrep/dep287

Bohensky, M. A., Jolley, D., Sundararajan, V., Evans, S., Pilcher, D. V., Scott, I., & Brand, C. A. (2010). Data linkage: a powerful research tool with potential problems. *BMC Health Serv Res, 10*, 346. doi:10.1186/1472-6963-10-346

Borenstein, M. (2009). *Introduction to meta-analysis*. Chichester, U.K.: John Wiley & Sons.

Boyd, N. F., Guo, H., Martin, L. J., Sun, L., Stone, J., Fishell, E., . . . Yaffe, M. J. (2007). Mammographic density and the risk and detection of breast cancer. *N Engl J Med, 356*(3), 227-236. doi:10.1056/NEJMoa062790

Braga, C., Negri, E., La Vecchia, C., Parazzini, F., Dal Maso, L., & Franceschi, S. (1996). Fertility treatment and risk of breast cancer. *Hum Reprod, 11*(2), 300-303.

Bray, F., McCarron, P., & Parkin, D. M. (2004). The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res, 6*(6), 229-239. doi:10.1186/bcr932

Breslow NE, D. N. (1987). *Statistical Methods in Cancer Research*. Oxford: Oxford University Press.

Brinton, L. A., Moghissi, K. S., Scoccia, B., Westhoff, C. L., & Lamb, E. J. (2005). Ovulation induction and cancer risk. *Fertil Steril, 83*(2), 261-274; quiz 525-266. doi:10.1016/j.fertnstert.2004.09.016

Brinton, L. A., Scoccia, B., Moghissi, K. S., Westhoff, C. L., Althuis, M. D., Mabie, J. E., & Lamb, E. J. (2004). Breast cancer risk associated with ovulation-stimulating drugs. *Hum Reprod, 19*(9), 2005-2013. doi:10.1093/humrep/deh371

Brinton, L. A., Scoccia, B., Moghissi, K. S., Westhoff, C. L., Niwa, S., Ruggieri, D., . . . Lamb, E. J. (2014). Long-term relationship of ovulation-stimulating drugs to breast cancer risk. *Cancer Epidemiol Biomarkers Prev, 23*(4), 584-593. doi:10.1158/1055-9965.EPI-13-0996

Brinton, L. A., Trabert, B., Shalev, V., Lunenfeld, E., Sella, T., & Chodick, G. (2013). In vitro fertilization and risk of breast and gynecologic cancers: a retrospective cohort study within the Israeli Maccabi Healthcare Services. *Fertil Steril, 99*(5), 1189-1196. doi:10.1016/j.fertnstert.2012.12.029

Brose, M. S., Rebbeck, T. R., Calzone, K. A., Stopfer, J. E., Nathanson, K. L., & Weber, B. L. (2002). Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. *J Natl Cancer Inst, 94*(18), 1365-1372.

Bulletti, C., Coccia, M. E., Battistoni, S., & Borini, A. (2010). Endometriosis and infertility. *J Assist Reprod Genet, 27*(8), 441-447. doi:10.1007/s10815-010-9436-1

Burkman, R. T., Tang, M. T., Malone, K. E., Marchbanks, P. A., McDonald, J. A., Folger, S. G., . . . Spirtas, R. (2003). Infertility drugs and the risk of breast cancer: findings from the National Institute of Child Health and Human Development Women's Contraceptive and Reproductive Experiences Study. *Fertil Steril, 79*(4), 844-851.

Bushnik, T., Cook, J. L., Yuzpe, A. A., Tough, S., & Collins, J. (2012). Estimating the prevalence of infertility in Canada. *Hum Reprod, 27*(3), 738-746. doi:10.1093/humrep/der465

Cahill, D. J., & Wardle, P. G. (2002). Management of infertility. *BMJ, 325*(7354), 28-32.

Calderon-Margalit, R., Friedlander, Y., Yanetz, R., Kleinhaus, K., Perrin, M. C., Manor, O., . . . Paltiel, O. (2009). Cancer risk after exposure to treatments for ovulation induction. *Am J Epidemiol, 169*(3), 365-375. doi:10.1093/aje/kwn318

Canadian Assisted Reproductive Technologies Registry (CARTR) Plus. (2017). *Preliminary treatment cycle data for 2016.* Paper presented at the Better Outcomes Registry & Network Ontario., Ottawa, Ontario.

Canadian Cancer Society's Advisory Committee on Cancer, & Statistics. (2017). *Canadian Cancer Statistics 2017*. Retrieved from Toronto, ON: cancer.ca/Canadian-CancerStatistics-2017-EN.pdf

Centers for Disease Control and Prevention: American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. (2016). *2014 Assisted Reproductive Technology National Summary Report.* . Retrieved from Atlanta (GA):

Clemons, M., & Goss, P. (2001). Estrogen and the risk of breast cancer. *N Engl J Med, 344*(4), 276-285. doi:10.1056/NEJM200101253440407

Colditz, G. A., & Bohlke, K. (2015). Preventing breast cancer now by acting on what we already know. *Npj Breast Cancer, 1*, 15009. doi:10.1038/npjbcancer.2015.9

Coldman, A., Phillips, N., Wilson, C., Decker, K., Chiarelli, A. M., Brisson, J., . . . Ahmad, R. (2014). Pan-Canadian study of mammography screening and mortality from breast cancer. *J Natl Cancer Inst, 106*(11). doi:10.1093/jnci/dju261

Collaborative Group on Hormonal Factors in Breast, C. (1996). Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet, 347*(9017), 1713-1727.

Collaborative Group on Hormonal Factors in Breast, C. (2012). Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol, 13*(11), 1141-1151. doi:10.1016/S1470-2045(12)70425-4

Collaborative Group on Hormonal Factors in Breast Cancer. (1997). Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. . *Lancet, 350*(9084), 1047-1059.

Cooper, A., & Tyrrell, F. (1824). *The lectures of Sir Astley Cooper ... on the principles and practice of surgery*. London,: T. and G. Underwood.

Coughlan, C., Fitzgerald, J., Milne, P., & Wingfield, M. (2010). Is it safe to prescribe clomiphene citrate without ultrasound monitoring facilities? *J Obstet Gynaecol, 30*(4), 393-396. doi:10.3109/01443611003646280

Cutting, R., Morroll, D., Roberts, S. A., Pickering, S., Rutherford, A., Bfs, & Ace. (2008). Elective single embryo transfer: guidelines for practice British Fertility Society and Association of Clinical Embryologists. *Hum Fertil (Camb), 11*(3), 131-146. doi:10.1080/14647270802302629

Dag, Z. O., & Dilbaz, B. (2015). Impact of obesity on infertility in women. *J Turk Ger Gynecol Assoc, 16*(2), 111-117. doi:10.5152/jtgga.2015.15232

Dalvai, M., & Bystricky, K. (2010). Cell cycle and anti-estrogen effects synergize to regulate cell proliferation and ER target gene expression. *PLoS One, 5*(6), e11011. doi:10.1371/journal.pone.0011011

Dartois, L., Fagherazzi, G., Baglietto, L., Boutron-Ruault, M. C., Delaloge, S., Mesrine, S., & Clavel-Chapelon, F. (2016). Proportion of premenopausal and postmenopausal breast cancers attributable to known risk factors: Estimates from the E3N-EPIC cohort. *Int J Cancer, 138*(10), 2415-2427. doi:10.1002/ijc.29987

Delaney, J. A., Daskalopoulou, S. S., Brophy, J. M., Steele, R. J., Opatrny, L., & Suissa, S. (2007). Lifestyle variables and the risk of myocardial infarction in the general practice research database. *BMC Cardiovasc Disord, 7*, 38. doi:10.1186/1471-2261-7-38

DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. *Control Clin Trials, 7*(3), 177-188.

Dion P, B. N. (2014). *Population Projections for Canada (2013 to 2063), Provinces and Territories (2013 to 2038): Technical Report on Methodology and Assumptions*. (91-620-X). Ottawa Retrieved from http://www.statcan.gc.ca/pub/91-620-x/2014001/chap03-eng.htm

Dor, J., Lerner-Geva, L., Rabinovici, J., Chetrit, A., Levran, D., Lunenfeld, B., . . . Modan, B. (2002). Cancer incidence in a cohort of infertile women who underwent in vitro fertilization. *Fertil Steril, 77*(2), 324-327.

dos Santos Silva, S., Wark, P. A., McCormack, V. A., Mayer, D., Overton, C., Little, V., . . . MacLean, A. B. (2009). Ovulation-stimulation drugs and cancer risks: a long-term follow-up of a British cohort. *Br J Cancer, 100*(11), 1824-1831. doi:10.1038/sj.bjc.6605086

Doyle, P., Maconochie, N., Beral, V., Swerdlow, A. J., & Tan, S. L. (2002). Cancer incidence following treatment for infertility at a clinic in the UK. *Hum Reprod, 17*(8), 2209-2213.

Dulberg CS, S. T. (1993). *The prevalence of infertility in Canada, 1991–1992: analysis of three national surveys*. Retrieved from Ottawa:

Eden, J. A., Place, J., Carter, G. D., Jones, J., Alaghband-Zadeh, J., & Pawson, M. E. (1989). The effect of clomiphene citrate on follicular phase increase in endometrial thickness and uterine volume. *Obstet Gynecol, 73*(2), 187-190.

Eerola, H., Heikkila, P., Tamminen, A., Aittomaki, K., Blomqvist, C., & Nevanlinna, H. (2005). Relationship of patients' age to histopathological features of breast tumours in BRCA1 and BRCA2 and mutation-negative breast cancer families. *Breast Cancer Res, 7*(4), R465-469. doi:10.1186/bcr1025

El-Bastawissi, A. Y., White, E., Mandelson, M. T., & Taplin, S. H. (2000). Reproductive and hormonal factors associated with mammographic breast density by age (United States). *Cancer Causes Control, 11*(10), 955-963.

Fei, C., Deroo, L. A., Sandler, D. P., & Weinberg, C. R. (2012). Fertility drugs and young-onset breast cancer: results from the Two Sister Study. *J Natl Cancer Inst, 104*(13), 1021-1027. doi:10.1093/jnci/djs255

Feng, Q., & O'Malley, B. W. (2014). Nuclear receptor modulation--role of coregulators in selective estrogen receptor modulator (SERM) actions. *Steroids, 90*, 39-43. doi:10.1016/j.steroids.2014.06.008

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., . . . Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer, 136*(5), E359-386. doi:10.1002/ijc.29210

Fiedler, K., & Ezcurra, D. (2012). Predicting and preventing ovarian hyperstimulation syndrome (OHSS): the need for individualized not standardized treatment. *Reprod Biol Endocrinol, 10*, 32. doi:10.1186/1477-7827-10-32

Filc, D., Davidovich, N., Novack, L., & Balicer, R. D. (2014). Is socioeconomic status associated with utilization of health care services in a single-payer universal health care system? *Int J Equity Health, 13*, 115. doi:10.1186/s12939-014-0115-1

Garcia, E., Diaz, O., Marti, R., Diez, Y., Gubern-Merida, A., Sentis, M., . . . Oliver, A. (2017). Local breast density assessment using reacquired mammographic images. *Eur J Radiol, 93*, 121-127. doi:10.1016/j.ejrad.2017.05.033

Gardner, D. K., Weissman, A., Howles, C. M., & Shoham, Z. (2018). *Textbook of assisted reproductive techniques* (Fifth edition. ed.). Boca Raton, FL: CRC Press.

Gauthier, E., Paoletti, X., Clavel-Chapelon, F., & group, E. N. (2004). Breast cancer risk associated with being treated for infertility: results from the French E3N cohort study. *Hum Reprod, 19*(10), 2216-2221. doi:10.1093/humrep/deh422

Gompel, A., Somai, S., Chaouat, M., Kazem, A., Kloosterboer, H. J., Beusman, I., . . . Rostene, W. (2000). Hormonal regulation of apoptosis in breast cells and tissues. *Steroids, 65*(10-11), 593-598.

Gotz, T., & Jones, C. (2017). Prioritization of Patients for Publicly Funded IVF in Ontario: A Survey of Fertility Centres. *J Obstet Gynaecol Can, 39*(3), 138-144. doi:10.1016/j.jogc.2016.11.011

Gunby, J., Daya, S., Fertility, I. V. F. D. G. o. t. C., & Andrology, S. (2005). Assisted reproductive technologies (ART) in Canada: 2001 results from the Canadian ART Register. *Fertil Steril, 84*(3), 590-599. doi:10.1016/j.fertnstert.2005.03.037

Hanson, K., Montgomery, P., Bakker, D., & Conlon, M. (2009). Factors influencing mammography participation in Canada: an integrative review of the literature. *Curr Oncol, 16*(5), 65-75.

Harron, K., Wade, A., Gilbert, R., Muller-Pebody, B., & Goldstein, H. (2014). Evaluating bias due to data linkage error in electronic healthcare records. *BMC Med Res Methodol, 14*, 36. doi:10.1186/1471-2288-14-36

Hemminki, K., Hemminki, O., Forsti, A., Sundquist, K., Sundquist, J., & Li, X. (2017). Surveillance Bias in Cancer Risk After Unrelated Medical Conditions: Example Urolithiasis. *Sci Rep, 7*(1), 8073. doi:10.1038/s41598-017-08839-5

Herrett, E., Gallagher, A. M., Bhaskaran, K., Forbes, H., Mathur, R., van Staa, T., & Smeeth, L. (2015). Data Resource Profile: Clinical Practice Research Datalink (CPRD). *Int J Epidemiol, 44*(3), 827-836. doi:10.1093/ije/dyv098

Higgins, J. P., & Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Stat Med, 21*(11), 1539-1558. doi:10.1002/sim.1186

Hofler, M. (2005). The Bradford Hill considerations on causality: a counterfactual perspective. *Emerg Themes Epidemiol, 2*, 11. doi:10.1186/1742-7622-2-11

Hou, N., Hong, S., Wang, W., Olopade, O. I., Dignam, J. J., & Huo, D. (2013). Hormone replacement therapy and breast cancer: heterogeneous risks by race, weight, and breast density. *J Natl Cancer Inst, 105*(18), 1365-1372. doi:10.1093/jnci/djt207

Howlader N, N. A., Krapcho M, Miller D, Bishop K, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds), . (2017). *SEER Cancer Statistics Review, 1975-2014*. Retrieved from Bethesda, MD: https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission

Huedo-Medina, T. B., Sanchez-Meca, J., Marin-Martinez, F., & Botella, J. (2006). Assessing heterogeneity in meta-analysis: Q statistic or I2 index? *Psychol Methods, 11*(2), 193-206. doi:10.1037/1082-989X.11.2.193

Human Fertilisation and Embryology Authority. (2016). *Fertility treatment 2014 - Trends and figures*. Retrieved from United Kingdom: https://www.hfea.gov.uk/.../hfea-fertility-treatment-2014-2016-trends-and-figures.pdf.

Hunlich, T., Trotnow, S., Mulz, D., & Kniewald, T. (1984). Hormonal and ultrasonic characteristics of 23 clomiphene citrate stimulated cycles resulting in pregnancies after in vitro fertilization and embryo transfer. *Arch Gynecol, 236*(1), 19-33.

Jain, A., Polotsky, A. J., Rochester, D., Berga, S. L., Loucks, T., Zeitlian, G., . . . Santoro, N. (2007). Pulsatile luteinizing hormone amplitude and progesterone metabolite excretion are reduced in obese women. *J Clin Endocrinol Metab, 92*(7), 2468-2473. doi:10.1210/jc.2006-2274

Javed, A., & Lteif, A. (2013). Development of the human breast. *Semin Plast Surg, 27*(1), 5-12. doi:10.1055/s-0033-1343989

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA Cancer J Clin, 61*(2), 69-90. doi:10.3322/caac.20107

Jensen, A., Sharif, H., Svare, E. I., Frederiksen, K., & Kjaer, S. K. (2007). Risk of breast cancer after exposure to fertility drugs: results from a large Danish cohort study. *Cancer Epidemiol Biomarkers Prev, 16*(7), 1400-1407. doi:10.1158/1055-9965.EPI-07-0075

Joint SOGC-CFAS. (2008). Guidelines for the number of embryos to transfer following in vitro fertilization No. 182, September 2006. *Int J Gynaecol Obstet, 102*(2), 203-216.

Jones, M. E., Schoemaker, M. J., Wright, L. B., Ashworth, A., & Swerdlow, A. J. (2017). Smoking and risk of breast cancer in the Generations Study cohort. *Breast Cancer Res, 19*(1), 118. doi:10.1186/s13058-017-0908-4

Joo, B. S., Park, S. H., An, B. M., Kim, K. S., Moon, S. E., & Moon, H. S. (2010). Serum estradiol levels during controlled ovarian hyperstimulation influence the pregnancy outcome of in vitro fertilization in a concentration-dependent manner. *Fertil Steril, 93*(2), 442-446. doi:10.1016/j.fertnstert.2009.02.066

Kallen, B., Finnstrom, O., Lindam, A., Nilsson, E., Nygren, K. G., & Olausson, P. O. (2011). Malignancies among women who gave birth after in vitro fertilization. *Hum Reprod, 26*(1), 253-258. doi:10.1093/humrep/deq307

Kallen, B., Finnstrom, O., Nygren, K. G., Otterblad Olausson, P., & Wennerholm, U. B. (2005). In vitro fertilisation in Sweden: obstetric characteristics, maternal morbidity and mortality. *BJOG, 112*(11), 1529-1535. doi:10.1111/j.1471-0528.2005.00745.x

Karapanou, O., & Papadimitriou, A. (2010). Determinants of menarche. *Reprod Biol Endocrinol, 8*, 115. doi:10.1186/1477-7827-8-115

Katz, D., Paltiel, O., Peretz, T., Revel, A., Sharon, N., Maly, B., . . . Allweis, T. (2008). Beginning IVF treatments after age 30 increases the risk of breast cancer: results of a case-control study. *Breast J, 14*(6), 517-522. doi:10.1111/j.1524-4741.2008.00641.x

Kessous, R., Davidson, E., Meirovitz, M., Sergienko, R., & Sheiner, E. (2016). The risk of female malignancies after fertility treatments: a cohort study with 25-year follow-up. *J Cancer Res Clin Oncol, 142*(1), 287-293. doi:10.1007/s00432-015-2035-x

Key, T. J., Appleby, P. N., Reeves, G. K., Roddam, A., Dorgan, J. F., Longcope, C., . . . Endogenous Hormones Breast Cancer Collaborative, G. (2003). Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J Natl Cancer Inst, 95*(16), 1218-1226.

Klein, J., & Sauer, M. V. (2001). Assessing fertility in women of advanced reproductive age. *Am J Obstet Gynecol, 185*(3), 758-770. doi:10.1067/mob.2001.114689

Kotsopoulos, J., Librach, C. L., Lubinski, J., Gronwald, J., Kim-Sing, C., Ghadirian, P., . . . Hereditary Breast Cancer Clinical Study, G. (2008). Infertility, treatment of infertility, and the risk of breast cancer among women with BRCA1 and BRCA2 mutations: a case-control study. *Cancer Causes Control, 19*(10), 1111-1119. doi:10.1007/s10552-008-9175-0

Kristiansson, P., Bjor, O., & Wramsby, H. (2007). Tumour incidence in Swedish women who gave birth following IVF treatment. *Human Reproduction, 22*(2), 421-426.

La Marca, A., Broekmans, F. J., Volpe, A., Fauser, B. C., Macklon, N. S., & Table, E. S. I. G. f. R. E.-.-A. R. (2009). Anti-Mullerian hormone (AMH): what do we still need to know? *Hum Reprod, 24*(9), 2264-2275. doi:10.1093/humrep/dep210

Lambe, M., Hsieh, C. C., Chan, H. W., Ekbom, A., Trichopoulos, D., & Adami, H. O. (1996). Parity, age at first and last birth, and risk of breast cancer: a population-based study in Sweden. *Breast Cancer Res Treat, 38*(3), 305-311.

Lauby-Secretan, B., Scoccianti, C., Loomis, D., Grosse, Y., Bianchini, F., Straif, K., & International Agency for Research on Cancer Handbook Working, G. (2016). Body Fatness and Cancer--Viewpoint of the IARC Working Group. *N Engl J Med, 375*(8), 794-798. doi:10.1056/NEJMsr1606602

Lee, C. H., Cook, S., Lee, J. S., & Han, B. (2016). Comparison of Two Meta-Analysis Methods: Inverse-Variance-Weighted Average and Weighted Sum of Z-Scores. *Genomics Inform, 14*(4), 173-180. doi:10.5808/GI.2016.14.4.173

Lee, H. J., Gallego-Ortega, D., Ledger, A., Schramek, D., Joshi, P., Szwarc, M. M., . . . Ormandy, C. J. (2013). Progesterone drives mammary secretory differentiation via RankL-mediated induction of Elf5 in luminal progenitor cells. *Development, 140*(7), 1397-1401. doi:10.1242/dev.088948

Lerner-Geva, L., Geva, E., Lessing, J. B., Chetrit, A., Modan, B., & Amit, A. (2003). The possible association between in vitro fertilization treatments and cancer development. *Int J Gynecol Cancer, 13*(1), 23-27.

Lerner-Geva, L., Keinan-Boker, L., Blumstein, T., Boyko, V., Olmar, L., Mashiach, S., . . . Lunenfeld, B. (2006). Infertility, ovulation induction treatments and the incidence of breast cancer--a historical prospective cohort of Israeli women. *Breast Cancer Res Treat, 100*(2), 201-212. doi:10.1007/s10549-006-9238-4

Lerner-Geva, L., Rabinovici, J., Olmer, L., Blumstein, T., Mashiach, S., & Lunenfeld, B. (2012). Are infertility treatments a potential risk factor for cancer development? Perspective of 30 years of follow-up. *Gynecol Endocrinol, 28*(10), 809-814. doi:10.3109/09513590.2012.671391

Lett, H. (1905). An Analysis of Ninety-nine Cases of Inoperable Carcinoma of the Breast treated by Oophorectomy. *Med Chir Trans, 88*, 147-189.

Ligibel, J. (2011). Obesity and breast cancer. *Oncology (Williston Park), 25*(11), 994-1000.

Losordo, D. W., & Isner, J. M. (2001). Estrogen and angiogenesis: A review. *Arterioscler Thromb Vasc Biol, 21*(1), 6-12.

Lu, L. J., Nishino, T. K., Johnson, R. F., Nayeem, F., Brunder, D. G., Ju, H., . . . Khamapirad, T. (2012). Comparison of breast tissue measurements using magnetic resonance imaging, digital mammography and a mathematical algorithm. *Phys Med Biol, 57*(21), 6903-6927. doi:10.1088/0031-9155/57/21/6903

Lundberg, F. E., Johansson, A. L., Rodriguez-Wallberg, K., Brand, J. S., Czene, K., Hall, P., & Iliadou, A. N. (2016). Association of infertility and fertility treatment with mammographic density in a large screening-based cohort of women: a cross-sectional study. *Breast Cancer Res, 18*(1), 36. doi:10.1186/s13058-016-0693-5

Lynch, H. T., Watson, P., Conway, T., Fitzsimmons, M. L., & Lynch, J. (1988). Breast cancer family history as a risk factor for early onset breast cancer. *Breast Cancer Res Treat, 11*(3), 263-267.

Macias, H., & Hinck, L. (2012). Mammary gland development. *Wiley Interdiscip Rev Dev Biol, 1*(4), 533-557. doi:10.1002/wdev.35

MacMahon, B., Cole, P., Lin, T. M., Lowe, C. R., Mirra, A. P., Ravnihar, B., . . . Yuasa, S. (1970). Age at first birth and breast cancer risk. *Bull World Health Organ, 43*(2), 209-221.

Makki, J. (2015). Diversity of Breast Carcinoma: Histological Subtypes and Clinical Relevance. *Clin Med Insights Pathol, 8*, 23-31. doi:10.4137/CPath.S31563

Malhotra, G. K., Zhao, X., Band, H., & Band, V. (2010). Histological, molecular and functional subtypes of breast cancers. *Cancer Biol Ther, 10*(10), 955-960.

Marmot, M. G., Altman, D. G., Cameron, D. A., Dewar, J. A., Thompson, S. G., & Wilcox, M. (2013). The benefits and harms of breast cancer screening: an independent review. *Br J Cancer, 108*(11), 2205-2240. doi:10.1038/bjc.2013.177

Martin JA, H. B., Osterman MJK, et al. . (2017). *Births: Final Data for 2015. National vital statistics report*. Retrieved from Hyattsville, MD:

Mascarenhas, M. N., Flaxman, S. R., Boerma, T., Vanderpoel, S., & Stevens, G. A. (2012). National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med, 9*(12), e1001356. doi:10.1371/journal.pmed.1001356

Mathur, R., Bhaskaran, K., Chaturvedi, N., Leon, D. A., vanStaa, T., Grundy, E., & Smeeth, L. (2014). Completeness and usability of ethnicity data in UK-based primary care and hospital databases. *J Public Health (Oxf), 36*(4), 684-692. doi:10.1093/pubmed/fdt116

McDonald, J. A., Goyal, A., & Terry, M. B. (2013). Alcohol Intake and Breast Cancer Risk: Weighing the Overall Evidence. *Curr Breast Cancer Rep, 5*(3). doi:10.1007/s12609-013-0114-z

McNutt, L. A., Wu, C., Xue, X., & Hafner, J. P. (2003). Estimating the relative risk in cohort studies and clinical trials of common outcomes. *Am J Epidemiol, 157*(10), 940-943.

Mikola, M., Hiilesmaa, V., Halttunen, M., Suhonen, L., & Tiitinen, A. (2001). Obstetric outcome in women with polycystic ovarian syndrome. *Hum Reprod, 16*(2), 226-229.

Modan, B., Ron, E., Lerner-Geva, L., Blumstein, T., Menczer, J., Rabinovici, J., . . . Lunenfeld, B. (1998). Cancer incidence in a cohort of infertile women. *Am J Epidemiol, 147*(11), 1038-1042.

Morch, L. S., Skovlund, C. W., Hannaford, P. C., Iversen, L., Fielding, S., & Lidegaard, O. (2017). Contemporary Hormonal Contraception and the Risk of Breast Cancer. *N Engl J Med, 377*(23), 2228-2239. doi:10.1056/NEJMoa1700732

Morissette, R. (2017). The surge of women in the workforce. *Canadian Megatrends.* Retrieved from http://www.statcan.gc.ca/pub/11-630-x/11-630-x2015009-eng.htm

Motluk, A. (2016). Ontario funds one cycle of IVF--while supplies last. *CMAJ, 188*(2), E32. doi:10.1503/cmaj.109-5210

National Cancer, I. (2005). Summary report: early reproductive events and breast cancer workshop. *Issues Law Med, 21*(2), 161-165.

National Cancer Institute. (2018). Breast Cancer Prevention (PDQ®)–Health Professional Version. Retrieved from https://www.cancer.gov/types/breast/hp/breast-prevention-pdq

National Collaborating Centre for Women's and Children's Health. (2013). *Fertility: Assessment and Treatment for People with Fertility Problems: Ovulation disorders*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK327781/

National Collaborating Centre for Women's and Children's Health (UK). (2013). *Fertility: Assessment and Treatment for People with Fertility Problems*. In NICE Clinical Guidelines, *8, Ovulation disorders*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK327781/

Nelson, L. R., & Bulun, S. E. (2001). Estrogen production and action. *J Am Acad Dermatol, 45*(3 Suppl), S116-124.

Nuttall, F. Q. (2015). Body Mass Index: Obesity, BMI, and Health: A Critical Review. *Nutr Today, 50*(3), 117-128. doi:10.1097/NT.0000000000000092

Ogden CL, C. M., Fryar CD, Flegal KM. (2015). *Prevalence of obesity among adults and youth: United States, 2011–2014*. Retrieved from Hyattsville, MD:

Olah, M. E., Gaisano, G., & Hwang, S. W. (2013). The effect of socioeconomic status on access to primary care: an audit study. *CMAJ, 185*(6), E263-269. doi:10.1503/cmaj.121383

Olsson, H., Baldetorp, B., Ferno, M., & Perfekt, R. (2003). Relation between the rate of tumour cell proliferation and latency time in radiation associated breast cancer. *BMC Cancer, 3*, 11.

Opatrny, L., Dell'Aniello, S., Assouline, S., & Suissa, S. (2008). Hormone replacement therapy use and variations in the risk of breast cancer. *BJOG, 115*(2), 169-175; discussion 175. doi:10.1111/j.1471-0528.2007.01520.x

Orgeas, C. C., Sanner, K., Hall, P., Conner, P., Holte, J., Nilsson, S. J., . . . Czene, K. (2009). Breast cancer incidence after hormonal infertility treatment in Sweden: a cohort study. *Am J Obstet Gynecol, 200*(1), 72 e71-77. doi:10.1016/j.ajog.2008.08.066

Ou, J., Xing, W., Li, Y., Xu, Y., & Zhou, C. (2015). Short versus Long Gonadotropin-Releasing Hormone Analogue Suppression Protocols in IVF/ICSI Cycles in Patients of Various Age Ranges. *PLoS One, 10*(7), e0133887. doi:10.1371/journal.pone.0133887

Pappo, I., Lerner-Geva, L., Halevy, A., Olmer, L., Friedler, S., Raziel, A., . . . Ron-El, R. (2008). The possible association between IVF and breast cancer incidence. *Ann Surg Oncol, 15*(4), 1048-1055. doi:10.1245/s10434-007-9800-2

Parakh, T. N., Hernandez, J. A., Grammer, J. C., Weck, J., Hunzicker-Dunn, M., Zeleznik, A. J., & Nilson, J. H. (2006). Follicle-stimulating hormone/cAMP regulation of aromatase gene expression requires beta-catenin. *Proc Natl Acad Sci U S A, 103*(33), 12435-12440. doi:10.1073/pnas.0603006103

Pasquali, R., Vicennati, V., Cacciari, M., & Pagotto, U. (2006). The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. *Ann N Y Acad Sci, 1083*, 111-128. doi:10.1196/annals.1367.009

Petro-Nustas, W., Norton, M. E., & al-Masarweh, I. (2002). Risk factors for breast cancer in Jordanian women. *J Nurs Scholarsh, 34*(1), 19-25.

Pike, M. C., Krailo, M. D., Henderson, B. E., Casagrande, J. T., & Hoel, D. G. (1983). 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. *Nature, 303*(5920), 767-770.

Pike, M. C., Spicer, D. V., Dahmoush, L., & Press, M. F. (1993). Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Rev, 15*(1), 17-35.

Platet, N., Cathiard, A. M., Gleizes, M., & Garcia, M. (2004). Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. *Crit Rev Oncol Hematol, 51*(1), 55-67. doi:10.1016/j.critrevonc.2004.02.001

Potashnik, G., Lerner-Geva, L., Genkin, L., Chetrit, A., Lunenfeld, E., & Porath, A. (1999). Fertility drugs and the risk of breast and ovarian cancers: Results of a long-term follow-up study. *Fertility and Sterility, 71*(5), 853-859.

Practice Committee of the American Society for Reproductive Medicine. (2016). Fertility drugs and cancer: a guideline. *Fertil Steril, 106*(7), 1617-1626. doi:10.1016/j.fertnstert.2016.08.035

Quaas, A., & Dokras, A. (2008). Diagnosis and treatment of unexplained infertility. *Rev Obstet Gynecol, 1*(2), 69-76.

Reed, B. G., & Carr, B. R. (2000). The Normal Menstrual Cycle and the Control of Ovulation. In L. J. De Groot, G. Chrousos, K. Dungan, K. R. Feingold, A. Grossman, J. M. Hershman, C. Koch, M. Korbonits, R. McLachlan, M. New, J. Purnell, R. Rebar, F. Singer, & A. Vinik (Eds.), *Endotext*. South Dartmouth (MA).

Reed, B. G., Ezzati, M., Babayev, S. N., Libby, V., Carr, B., & Bukulmez, O. (2015). Use of clomiphene citrate in minimal ovarian stimulation cycles negatively impacts endometrial thickness, independent of peak estradiol levels: an argument for a freeze-all approach. *Fertility and Sterility, 104*(3), e170. doi:10.1016/j.fertnstert.2015.07.526

Rees, D. A., Jenkins-Jones, S., & Morgan, C. L. (2016). Contemporary Reproductive Outcomes for Patients With Polycystic Ovary Syndrome: A Retrospective Observational Study. *J Clin Endocrinol Metab, 101*(4), 1664-1672. doi:10.1210/jc.2015-2682

Reeves, D., Springate, D. A., Ashcroft, D. M., Ryan, R., Doran, T., Morris, R., . . . Kontopantelis, E. (2014). Can analyses of electronic patient records be independently and externally validated? The effect of statins on the mortality of patients with ischaemic heart disease: a cohort study with nested case-control analysis. *BMJ Open, 4*(4), e004952. doi:10.1136/bmjopen-2014-004952

Reigstad, M. M., Larsen, I. K., Myklebust, T. A., Robsahm, T. E., Oldereid, N. B., Omland, A. K., . . . Storeng, R. (2015). Cancer risk among parous women following assisted reproductive technology. *Hum Reprod, 30*(8), 1952-1963. doi:10.1093/humrep/dev124

Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, T. C. C., 2014, . In.

Ricci, E., Parazzini, F., Negri, E., Marsico, S., & La Vecchia, C. (1999). Fertility drugs and the risk of breast cancer. *Hum Reprod, 14*(6), 1653-1655.

Rossing, M. A., Daling, J. R., Weiss, N. S., Moore, D. E., & Self, S. G. (1996). Risk of breast cancer in a cohort of infertile women. *Gynecol Oncol, 60*(1), 3-7.

Roumen, F. J. (1997). [Decreased quality of cervix mucus under the influence of clomiphene: a meta-analysis]. *Ned Tijdschr Geneeskd, 141*(49), 2401-2405.

Sam, S. (2007). Obesity and Polycystic Ovary Syndrome. *Obes Manag, 3*(2), 69-73. doi:10.1089/obe.2007.0019

Samavat, H., & Kurzer, M. S. (2015). Estrogen metabolism and breast cancer. *Cancer Lett, 356*(2 Pt A), 231-243. doi:10.1016/j.canlet.2014.04.018

Schafer, J. L. (1999). Multiple imputation: a primer. *Stat Methods Med Res, 8*(1), 3-15. doi:10.1177/096228029900800102

Schinziger A. (1889). Uber Carcinoma Mammae. *Verhandlungen der Gesellschaft fuer Orthopaedische Chirurgie, 18*(28

).

Schwartz, L. B., Brezinski, A., & Laufer, N. (1993). The effect of clomiphene citrate isomers on human granulosa-lutein cells in culture. *Gynecol Endocrinol, 7*(4), 229-233.

Sergentanis, T. N., Diamantaras, A. A., Perlepe, C., Kanavidis, P., Skalkidou, A., & Petridou, E. T. (2014). IVF and breast cancer: a systematic review and meta-analysis. *Hum Reprod Update, 20*(1), 106-123. doi:10.1093/humupd/dmt034

Siegelmann-Danieli, N., Tamir, A., Zohar, H., Papa, M. Z., Chetver, L. L., Gallimidi, Z., . . . Kuten, A. (2003). Breast cancer in women with recent exposure to fertility medications is associated with poor prognostic features. *Annals of Surgical Oncology, 10*(9), 1031-1038.

Sprague, B. L., Trentham-Dietz, A., Terry, M. B., Nichols, H. B., Bersch, A. J., & Buist, D. S. (2008). Fertility drug use and mammographic breast density in a mammography

screening cohort of premenopausal women. *Cancer Epidemiol Biomarkers Prev, 17*(11), 3128-3133. doi:10.1158/1055-9965.EPI-08-0503

Spruance, S. L., Reid, J. E., Grace, M., & Samore, M. (2004). Hazard ratio in clinical trials. *Antimicrob Agents Chemother, 48*(8), 2787-2792. doi:10.1128/AAC.48.8.2787-2792.2004

Steinkampf, M. P., Mendelson, C. R., & Simpson, E. R. (1987). Regulation by follicle-stimulating hormone of the synthesis of aromatase cytochrome P-450 in human granulosa cells. *Mol Endocrinol, 1*(7), 465-471. doi:10.1210/mend-1-7-465

Stewart, L. M., & Hart, R. (2015). Long-term cancer risks in women after treatment with IVF: do we have any answers yet? *Womens Health (Lond), 11*(1), 7-10. doi:10.2217/whe.14.58

Stewart, L. M., Holman, C. D., Hart, R., Bulsara, M. K., Preen, D. B., & Finn, J. C. (2012). In vitro fertilization and breast cancer: is there cause for concern? *Fertil Steril, 98*(2), 334-340. doi:10.1016/j.fertnstert.2012.04.019

Sutcliffe D, L. H., Hutton J, Stokes T. (2012). NICE and the quality and outcomes framework (QOF) 2009–2011. *Quality in Primary Care 20*(1), 47-55.

Tata, J. R. (2005). One hundred years of hormones. *EMBO Rep, 6*(6), 490-496. doi:10.1038/sj.embor.7400444

Terry, K. L., Willett, W. C., Rich-Edwards, J. W., & Michels, K. B. (2006). A prospective study of infertility due to ovulatory disorders, ovulation induction, and incidence of breast cancer. *Arch Intern Med, 166*(22), 2484-2489. doi:10.1001/archinte.166.22.2484

Terry, P. D., Miller, A. B., & Rohan, T. E. (2002). Cigarette smoking and breast cancer risk: a long latency period? *Int J Cancer, 100*(6), 723-728. doi:10.1002/ijc.10536

Tulandi, T., King, L., & Zelkowitz, P. (2013). Public funding of and access to in vitro fertilization. *N Engl J Med, 368*(20), 1948-1949. doi:10.1056/NEJMc1213687

Turkoz, F. P., Solak, M., Petekkaya, I., Keskin, O., Kertmen, N., Sarici, F., . . . Altundag, K. (2013). Association between common risk factors and molecular subtypes in breast cancer patients. *Breast, 22*(3), 344-350. doi:10.1016/j.breast.2012.08.005

Tyrer, J., Duffy, S. W., & Cuzick, J. (2004). A breast cancer prediction model incorporating familial and personal risk factors. *Stat Med, 23*(7), 1111-1130. doi:10.1002/sim.1668

Ursin, G., Bernstein, L., Wang, Y., Lord, S. J., Deapen, D., Liff, J. M., . . . Spirtas, R. (2004). Reproductive factors and risk of breast carcinoma in a study of white and African-American women. *Cancer, 101*(2), 353-362. doi:10.1002/cncr.20373

Vahratian, A. (2009). Prevalence of overweight and obesity among women of childbearing age: results from the 2002 National Survey of Family Growth. *Matern Child Health J, 13*(2), 268-273. doi:10.1007/s10995-008-0340-6

van den Belt-Dusebout, A. W., Spaan, M., Lambalk, C. B., Kortman, M., Laven, J. S., van Santbrink, E. J., . . . van Leeuwen, F. E. (2016). Ovarian Stimulation for In Vitro Fertilization and Long-term Risk of Breast Cancer. *JAMA, 316*(3), 300-312. doi:10.1001/jama.2016.9389

Venn, A., Watson, L., Bruinsma, F., Giles, G., & Healy, D. (1999). Risk of cancer after use of fertility drugs with in-vitro fertilisation. *Lancet, 354*(9190), 1586-1590. doi:10.1016/S0140-6736(99)05203-4

Venn, A., Watson, L., Lumley, J., Giles, G., King, C., & Healy, D. (1995). Breast and ovarian cancer incidence after infertility and in vitro fertilisation. *Lancet, 346*(8981), 995-1000.

Wanebo, C. K., Johnson, K. G., Sato, K., & Thorslund, T. W. (1968). Breast cancer after exposure to the atomic bombings of Hiroshima and Nagasaki. *N Engl J Med, 279*(13), 667-671. doi:10.1056/NEJM196809262791301

Weiss, N. S., Nahuis, M., Bayram, N., Mol, B. W., Van der Veen, F., & van Wely, M. (2015). Gonadotrophins for ovulation induction in women with polycystic ovarian syndrome. *Cochrane Database Syst Rev*(9), CD010290. doi:10.1002/14651858.CD010290.pub2

WHO International Agency for Research on Cancer. (2014). *Breast Cancer: Estimated Incidence, Mortality and Prevalence Worldwide in 2012*. Retrieved from http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx

Wilkes, S., Chinn, D. J., Murdoch, A., & Rubin, G. (2009). Epidemiology and management of infertility: a population-based study in UK primary care. *Fam Pract, 26*(4), 269-274. doi:10.1093/fampra/cmp029

World Health Organization Global Database on Body Mass Index Retrieved from http://apps.who.int/bmi/index.jsp?introPage=intro_3.html.

Yager, J. D., & Davidson, N. E. (2006). Estrogen carcinogenesis in breast cancer. *N Engl J Med, 354*(3), 270-282. doi:10.1056/NEJMra050776

Yli-Kuha, A. N., Gissler, M., Klemetti, R., Luoto, R., & Hemminki, E. (2012). Cancer morbidity in a cohort of 9175 Finnish women treated for infertility. *Hum Reprod, 27*(4), 1149-1155. doi:10.1093/humrep/des031

Yue, W., Yager, J. D., Wang, J. P., Jupe, E. R., & Santen, R. J. (2013). Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis. *Steroids, 78*(2), 161-170. doi:10.1016/j.steroids.2012.11.001

Zegers-Hochschild, F., Adamson, G. D., de Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., . . . World Health, O. (2009). The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. *Hum Reprod, 24*(11), 2683-2687. doi:10.1093/humrep/dep343

Zhivotovsky, B., & Kroemer, G. (2004). Apoptosis and genomic instability. *Nat Rev Mol Cell Biol, 5*(9), 752-762. doi:10.1038/nrm1443

Zhuang, L. Z., Adashi, E. Y., & Hsuch, A. J. (1982). Direct enhancement of gonadotropin-stimulated ovarian estrogen biosynthesis by estrogen and clomiphene citrate. *Endocrinology, 110*(6), 2219-2221. doi:10.1210/endo-110-6-2219

Zreik, T. G., Mazloom, A., Chen, Y., Vannucci, M., Pinnix, C. C., Fulton, S., . . . Dabaja, B. (2010). Fertility drugs and the risk of breast cancer: a meta-analysis and review. *Breast Cancer Res Treat, 124*(1), 13-26. doi:10.1007/s10549-010-1140-4