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Tobacco smoke exposures and fertility-related outcomes among females seeking fertility care, and the interaction with N-Acetyltransferase 2 (NAT2)..

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TOBACCO SMOKE EXPOSURES AND FERTILITY-RELATED OUTCOMES AMONG FEMALES SEEKING FERTILITY CARE, AND INTERACTION WITH N-ACETYLTRANSFERASE 2 (NAT2)

By

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B.A., Bellarmine University, 2011
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ABSTRACT

TOBACCO SMOKE EXPOSURES AND FERTILITY-RELATED OUTCOMES AMONG FEMALES SEEKING FERTILITY CARE, AND INTERACTION WITH N-ACETYLTRANSFERASE 2 (NAT2)

T'shura Ali

April 16th, 2020

Cigarette smoke contains thousands of harmful substances and is one of the leading preventable causes of mortality and morbidity in the United States. Past studies examining tobacco smoke exposures on fecundability and pregnancy outcomes are inconsistent. NAT2 is an important enzyme in the metabolism of xenobiotic substances found within tobacco smoke. This preconception cohort study examines associations between active smoking and secondhand smoke exposure (SHSe) on fecundability and spontaneous abortion (SA), and explores a possible interaction with NAT2 acetylator status.

A total of 223 women seeking fertility care were followed for up to 2.3 years. Preconception tobacco smoke exposures were collected by questionnaires and verified by urinary cotinine. SHSe at home and work was measured using the questionnaire (never, rarely (once/week), often (1-6 times/week), daily for each location) and then combined and categorized as low or high SHSe. NAT2 was genotyped to determine acetylator status (rapid vs slow). Pregnancy outcomes (SA vs live birth) were collected on 72 women. Cox proportional hazards regression was used to estimate fecundability ratios (FR) and 95% confidence intervals (CI), and logistic regression to estimate odds ratios (OR) and 95% CIs for the association of active smoking and SHSe on fecundability and

SA, respectively. Full models were adjusted for age, BMI, assisted conception, gravidity, marital status, alcohol use and race.

Overall, no significant effect of tobacco smoke exposure on fecundability was established. Though statistically insignificant, the effect of smoking on fecundability was stronger among slow NAT2 acetylators. Smokers (OR: 6.28; 95% CI 1.31, 37.9) and nonsmokers with high SHSe (OR: 3.20; 95% CI 0.87, 12.7) had increased odds of SA (ptrend= 0.02), compared to nonsmokers with low SHSe. Among nonsmokers, women with high SHSe had higher odds of SA (OR: 4.30; 95% CI 1.14, 19.1) than women to low SHSe. No significant interaction with NAT2 was reported. Despite wide CIs, results suggest that active smoking and high levels of SHSe may increase in the risk of SA among women seeking fertility care. This dissertation has clinical implications for patient care, and points to biological mechanisms by which tobacco smoke may affect fertility and pregnancy outcomes.

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I. INTRODUCTION

Infertility is a growing health problem, in the United States (U.S.) and worldwide. In the last 10 years, the U.S. has experienced increases in infertility and impaired fecundity, along with a greater use of infertility services (1,2). Infertility is not just the absence of children; it is classified as a disease of the reproductive system and affects all aspects of health. The impact of infertility can start with undiagnosed health conditions and lead to severe financial, and emotional and psychological burdens (3).

Infertility, defined as the inability to conceive after at least 12 months of trying, can affect both men and women (3,4). Approximately one third of all infertility cases are due to male factors and one third due to female factors; unexplained or "idiopathic" infertility, as well as both male and female factors account for the last third (5). The dissertation will focus primarily on female factors of infertility. There are several etiologies/factors of female infertility and which are grouped into ovarian, tubal, uterine, cervical and other factors such as autoimmune disorders (6).

Infertility treatment depends on the type of infertility diagnosis. Treatments can range from education on changing lifestyle behaviors, to ovarian induction medications, to assisted reproductive therapies (ART). Medically assisted reproduction (MAR) is the term used for all therapies including ovulation induction (OI), intrauterine insemination (IUI), and ART, which in itself includes in vitro fertilization (IVF) with or without

intracytoplasmic sperm injection (ICSI). In the U.S., approximately 1.7% of all infants born in 2017 were conceived through ART services (7). While infertility and the use of MAR services are increasing, the risk of adverse pregnancy outcomes has been shown to be higher among assisted compared to natural conceptions. Several studies have found significantly higher rates of spontaneous abortion (SA) among ART-assisted conceptions, compared to the general population (8).

There are also several important risk factors that have been associated with infertility and/or SA. These include older age, genetic factors, menstrual cycle irregularities, sexually transmitted infections, extreme (very high and very low) levels of stress, extreme levels of body mass index (BMI), extreme levels of exercise, diet, heavy caffeine use, heavy alcohol consumption and tobacco smoke exposure (9). Prior pregnancy loss and certain medication use are included with the previous list as risk factors for SA (10).

Tobacco smoke exposure is one of the leading preventable causes of mortality and morbidity in the U.S. and has been found to be associated with longer time to conception, lower conception rates, and adverse pregnancy outcomes such as SA (11). While the U.S. Surgeon General report supported the association of active, current smoking on conception, it stated that there was less evidence of an association among women using MAR. The report also stated that there was suggestive but insufficient evidence to infer an association between current smoking and SA (12). In addition, studies examining the effect of secondhand smoke exposures on fertility and fertility-related outcomes are inconclusive and inconsistent. Many studies are subject to bias because of their retrospective design and reliance on self-reported information about smoking. In addition,

the majority of studies did not take all potential confounding factors into account. Finally, almost no studies explored possible gene-environmental interactions.

N-acetyltransferase 2 is an enzyme used in the metabolism and detoxification of xenobiotic substances, including therapeutic drugs and exogenous chemicals such as the ones found in tobacco smoke (13). Genetic variation in the NAT2 gene results in different levels of acetylation, commonly grouped into rapid and slow acetylation phenotypes. NAT2 acetylator status may modify the metabolism of environmental toxins and therapeutic medicines. NAT2 acetylator status has also been associated with differences in cancer risk and may modify the effect of smoking on some cancers (14). Few studies have explored how NAT2 can modify the association of smoking and fertility. To date, only one study has found a significant interaction between NAT2 and smoking on female fertility (15), while two studies on idiopathic male infertility showed contradictory results (16,17). These studies have emphasized the importance of further exploring gene-environmental interactions on fertility outcomes.

This dissertation explores the effects of active and secondhand tobacco smoke exposures on fertility-related outcomes and examines the interaction of NAT2 with tobacco smoke exposure among women seeking fertility counselling and treatment.

II. OBJECTIVE, SPECIFIC AIMS AND HYPOTHESES

The primary objective of the study was to assess the effect of active smoking (current and lifetime), secondhand smoke exposure (recent and in the past year) and the combined effect of both exposures on fecundability and probability of spontaneous abortion. Effect modification by NAT2 acetylator status on conception and spontaneous abortion was also examined. The potential for selection bias due to loss to follow-up was investigated. Analyses were done using (1) all women and their follow-up time, whether or not they used medically assisted reproduction (MAR); (2) MAR treatment cycles only; and (3) all other follow-up time, which is termed natural conception (NC) follow-up time, for the purposes of this dissertation. The populations are not mutually exclusive.

The specific aims of the study are as follows:

Specific Aim 1: Examine the association between **tobacco smoke exposures** (active and passive) and **fecundability** after fertility counselling among three populations: all follow-up time; medically assisted reproduction (MAR) cycles; and natural conception (NC) follow-up time.

a. Examine the effect of **active smoking** (current and lifetime) on fecundability for all populations. *Hypothesis: Active smoking (current and lifetime) is associated with reduced fecundability.*

b. Examine the effect of **secondhand smoke exposure** (recent and in the past year) on fecundability for all populations. *Hypothesis: Secondhand smoke exposure (recent and past year) is associated with reduced fecundability.*

c. Examine the **combined effect of active smoking with secondhand smoke exposure** on fecundability for all populations. *Hypothesis: There is a dose-response relationship, in that smokers will have the lowest fecundability, and non-smokers with high SHS exposure will have reduced fecundability when compared with non-smokers with low SHS exposure.*

d. Examine the **interaction of active smoking with NAT2 acetylator status** for the measures estimated in aims 1a above. *Hypothesis: There is a synergistic effect between active smoking and slow acetylation status, such that active smokers who are also slow acetylators will have markedly reduced fecundability.*

Specific Aim 2: Examine the association between **tobacco smoke exposures** (active and passive) and **pregnancy outcomes** among individuals who conceived, after fertility counseling.

a. Examine the effect of **active smoking** (current and lifetime) on pregnancy outcomes (spontaneous abortion vs live birth) among all conceptions. *Hypothesis: Active smoking is associated with higher probability of spontaneous abortion.*

b. Examine the effect of **secondhand smoke exposure** (recent and in the past year) on pregnancy outcomes (spontaneous abortion vs live birth) among all conceptions. *Hypothesis: Higher exposure to secondhand smoke exposure (recent and in the past year) is associated with a higher probability of spontaneous abortion.*

c. Examine the **combined effect of active smoking with secondhand smoke exposure** on pregnancy outcomes (miscarriage vs live birth) after fertility counseling. *Hypothesis: There is a dose-response relationship, in that smokers will have the highest probability of spontaneous abortion; non-smokers with high SHS exposure will have intermediate probability; and non-smokers with low SHS exposure will have the lowest probability of spontaneous abortion.*

d. Examine the **interaction of active smoking with NAT2 acetylator status** for the measures estimated in aims 2a and 2b, above. *Hypothesis: Active smokers who are also slow acetylators will have a markedly increased probability of spontaneous abortion (higher than expected when examining each risk factor alone).*

Specific Aim 3: Evaluate the potential for **selection bias due to loss to follow-up** in the LOUSSI Study.

a. Evaluate the potential for **selection bias due to loss to follow-up** by comparing characteristics of participants lost to follow-up to participants

with complete follow-up. *Hypothesis: Characteristics of those lost to follow-up are similar to those with complete follow-up.*

b. If there is evidence for selection bias due to loss to follow-up, **apply inverse probability weighting to adjust for selection bias**. *Hypothesis: If there is no selection bias due to loss to follow-up, there will be no differences in the odds/hazard ratio estimates when compared to the initial analysis.*

III. BACKGROUND AND LITERATURE REVIEW

SECTION A - Infertility

The World Health Organization's International Committee for Monitoring Assisted Reproductive Technologies (WHO-ICMART), along with the Center for Disease Control and Prevention (CDC) describes infertility as a disease of the reproductive system defined by the inability to attain a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner (3,4). Other fertility-related terms include subfertility, sterility, fecundity, impaired fecundity and fecundability. Most are used interchangeably, hence the need for more formal and universal definitions to accurately compare studies.

In 2017, WHO-ICMART revised and expanded their 2009 edition of the International Glossary on Infertility and Fertility Care, from 87 to 283 terminologies (4). They define fertility as the capacity to establish a clinical pregnancy, sterility as a permanent state of infertility and state that subfertility should be used interchangeably with infertility. However, one article defined subfertility as any form of reduced fertility in couples unsuccessfully trying to get pregnant, not specifying a time period (18). Fecundity, on the other hand, is not just the capacity to conceive, but to also carry the

pregnancy to term, which is also known as the reproductive potential (19). The CDC states that infertility is specific only to married couples and deals only with issues getting pregnant; whereas impaired fecundity refers generally to all women regardless of marital or relationship status, and deals with problems with getting pregnant and carrying a baby to term (19). Time to pregnancy or conception (TTC) is the number of months or menstrual cycles taken to establish a pregnancy and fecundability is the probability of conception per month or menstrual cycle (4).

Infertility is commonly classified into two main groups: primary infertility and secondary infertility, which can then be sub-divided by gender. Firstly, primary infertility for females is diagnosed if there has not been a clinical pregnancy and the infertility criteria is met. For males, primary infertility is defined when there was never initiation of a clinical pregnancy and by the infertility criteria (3). Female secondary infertility is defined by the inability to establish a clinical pregnancy following a previous pregnancy and similarly, male secondary infertility is the inability to initiate a clinical pregnancy after successfully initiating one before (4).

The highest probability of conception among the general population occurs 2 days before ovulation of the first cycle of intercourse without contraception use and is approximately 30% (20). This probability decreases slightly over time/cycles; one article showed that the cumulative probability of conceiving within the first three cycles of unprotected intercourse is around 30%, then 75% within six cycles and almost 90% within 12 months (20). Highly fertile couples will conceive in the first cycles of attempt and others will still naturally conceive even after 12 months (20).

Epidemiology

Infertility is known to be a global public health issue; however, global prevalence rates are very difficult and tedious to calculate due to the inconsistency within the literature. Studies use different definitions, for example the length of time actively trying to conceive using either 12 months, 24 months or 5 years. Also, the population included in the definition varies from including females, couples, individuals or married women. The lack of population-based studies and differences in definition and methodologies adds to the difficulty in estimating and comparing global infertility rates (21). However, in 2010 the WHO published a study on global infertility trends using 277 surveys from 190 different countries.

The prevalence of primary infertility among women aged 20 to 44 years was calculated at 1.9%; and secondary infertility, which was defined inability to have a second live birth, was calculated at 10.5% (22). They defined infertility as the inability to have a live birth over a 5-year period and was based on marriage/union status, desire for child and no contraception use. Worldwide, a small decrease from 1990 to 2010 in primary infertility (1.6% to 1.5%) and in secondary infertility (3.9% to 3.0%) was reported. The absolute number of couples impacted by infertility, though has increased from 42 million in 1990, to 48.5 million in 2010 due to accelerated population growth (22). The WHO stated that there was a significant increase to 121 million when the infertility time period was cut down to 2 years instead of 5. The overall global prevalence of infertility was calculated to range from 8% to 12% of couples (22). It was also noted that developing regions and countries such as sub-Saharan Africa, the Middle East, North Africa, South Asia, Central and South Asia, and Eastern Europe experienced

disproportionately higher infertility rates up to 30% (21). This may be due to higher secondary infertility rates which can result from infections from unsafe abortions and poor post-partum or maternity care (21). The global infertility prevalence from this study and other studies that definition of infertility only include married women are highly underestimated.

Recently in December 2019, authors published estimates of the global burden of infertility, using results from a 2017 global burden of disease study among 195 countries. They found that worldwide, there had been a 0.37% increase annually in age-standardized infertility prevalence rates for females and a slightly lower increase, 0.29% for males (23). These increases were maintained over all sociodemographic index countries.

In the U.S., the National Center for Health Statistics used data from National Survey of Family Growth (NSFG) and showed that percentages of married women aged 15 to 44 years with infertility were 7.4%, 6.0%, 6.7% and 8.8 % in 2002, 2006-2010, 2011-2015 and 2015-2017, respectively (1,2). This increasing trend was retained after women were categorized into four age groups (15-29, 30-34, 35-39, 40-44) (1,2). A similar trend for impaired fecundity, which takes into account all women into account regardless of their marital status showed 11.8%, 10.9%, 12.1% and 13.1 % in 2002, 2006-2010, 2011-2015 and 2015-2017, respectively (1,2). A similar increasing trend was shown with age. In 2013, one study estimated the prevalence of couple infertility from a male's perspective using the 2002 NSFG data. Males estimated the prevalence of couple's infertility at 12.0% (95% CI: 7.0, 23.2), which was found to be consistent with female reporting in prospective cohort studies (24).

Women and men can both endure emotional and psychological stress such as depression, discrimination and ostracism when dealing with infertility (21,22). The impact of infertility is all encompassing, ranging from the unexpected medical diagnoses to the financial burden of medical services and health disparities to access of care. Infertility has also been shown to be associated with adverse pregnancy outcomes, which can consequently affect the onset of adult diseases later in life and quality of life for the individuals (3).

Causes, Risk Factors and Biological Mechanisms

There are a number of well-defined risk factors that have been studied and found to increase the odds of being infertile among women (9). These factors can be non-modifiable such as age (25–27), irregular menstrual cycles (28–31), and some genetic factors (32–34). Then there are several modifiable lifestyle factors that can affect fertility including diet (35–37), caffeine (38–40), alcohol consumption (29–31), body mass index (41–43), exercise (41,43,44), sexually transmitted infections (45,46), and stress and psychological state (47,48). Some of these important risk factors and causes, along with their biological mechanisms are outlined below. Tobacco smoke specifically, and its possible biological mechanisms on fertility are presented in the next section.

Conception is a result of a multistep process that has to meet every criteria to be successful. Firstly, a woman must ovulate which means an egg is released from one of her ovaries and travels along the fallopian tube to the site of fertilization. A man's sperm must also travel up the uterus to fertilize the egg. After successful fertilization, the fertilized egg known as the zygote then travels down the fallopian tube to the uterus. The

zygote will proliferate into an embryo, which develops into a blastocyte and will then implant itself into the uterus lining. Disruptions at any stage can lead to infertility issues (5).

Among all infertility cases, female factors account for approximately 35% and male factors account for 35% (5,49). The majority of the remaining 30% of cases do not meet the criteria for any known causes of infertility and are diagnosed with unexplained infertility (5). Female infertility has several etiologies that can be grouped into categories: ovulation dysfunction, tubal obstruction, uterine abnormalities, cervical factors and other factors such as autoimmune disorders and genetic factors (6). The different categorizations of etiologies also help to differentiate the specific type of infertility diagnosis.

Ovulatory Dysfunction

Ovulatory dysfunction is one of the common causes of female infertility, accounting for over 30% of cases (49). It can manifest in a variety of forms including anovulation (absence of ovulation), oligoovulation (irregular ovulation), ovarian aging and diminished ovarian reserve (DOR) (5). Ovarian reserve is the quantity and quality of the oocytes within the ovaries and is highly correlated to reproductive potential (5). The main causes of DOR are aging, obesity, cigarette smoking, genetic abnormalities (Fragile X and other X chromosome abnormalities), aggressive treatments (radiation) and ovarian surgery (for endometriosis). Other causes of DOR are unknown and are termed idiopathic (50). Some women with low ovarian reserve may respond to ovarian stimulation with a poor follicular development and may need more aggressive management; however, some

women with higher ovarian reserve may experience ovarian hyperstimulation syndrome (OHSS) that can also lead to adverse fertility outcomes (5,50). One retrospective cohort study in 2015 showed a significant increase in DOR from 19% in 2004 to 26% in 2011 (51). However, few studies have reported that DOR is not an accurate predictor of poor ovarian response (51).

Ovarian aging is a term used to describe the natural decline in the quantity and quality of eggs due to normal aging (5,49). The biological mechanisms behind ovarian aging are not fully understood, however, it is established that as the follicle pool diminishes with age, the odds of chromosomal abnormalities increases (49). While ovarian aging is a natural process, DOR can also affect younger women. Ovarian aging and DOR are quantified through Antral Follicle Count (AFC) and different reproductive hormones such as Follicle-Stimulating Hormone (FSH), Antimüllerian Hormone (AMH), luteinizing hormone (LH) and estradiol (49). One prospective fecundability study showed the percentage of infertility increased with woman's age: 8% among 19 through 26-year-olds, 13%-14% for 27 through 34-year-olds and 18% among 35 through 39-year-olds (26). Lower fecundability rates have also been seen among aging follicles with linear decline in fecundability and increasing female age (27). Primary Ovary Insufficiency (POI) is another disorder, where the ovaries stop producing hormones and releasing eggs, and experience either anovulation or oligoovulation (52). It is a common condition that increases with age and can induce premature or early menopause (53). One study showed a 10-fold increase every decade for the prevalence of POI, from 0.01% among women younger than 20 years to 0.1% in women under 30 years of age and 1% among women younger than 40 years (54).

Amenorrhea (absence of bleeding) and oligomenorrhea (irregular or extended menstrual cycle bleeding) are also related factors in anovulation infertility (49). The menstrual cycle includes several stages that prepare the body for conception. Irregularities or disruption at any stage can lead to infertility. Two recent studies have shown that any deviation from normal or the average menstrual cycle characteristics such as longer or shorter menstrual cycle lengths, later or earlier onset of menarche and shorter or longer bleeding duration have been associated with reduced fecundability (30,31).

Other diseases associated with anovulation include thyroid disease, and hypothalamic and pituitary dysfunction which controls the production of hormones (5,49). Functional hypothalamic amenorrhea (FHA) is one the most common causes of secondary amenorrhea and is associated with chronic stress and psychiatric disorders like depression (55). Studies done both in the general and infertile population have shown that psychological distress including chronic stress have been shown to increase odds of DOR, affect menstrual cycle characteristics, lower conception rates and increase adverse pregnancy outcomes (47,48,56).

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most common cause of anovulation and affect 6 to 12% (~ 5 million) of reproductive aged women in the U.S. (57). Among anovulatory infertility, PCOS accounts for 80% to 90% of cases (58). It is characterized by oligomenorrhea, obesity, insulin-resistance and an overproduction of androgen, which increases LH and lowers FSH. Hormonal imbalances have been shown to obstruct the development and maturation of follicles and ovulation, leading to issues with fertility and

fertility treatments (5). PCOS can also arise from endocrine, genetic, lifestyle and environmental factors.

Anovulation infertility is commonly found in both overweight (BMI ≥ 30 kg/m2) and severely underweight women (BMI < 17 kg/m2) (49). One case-control study compared cases of anovulatory infertility to fertile controls and found that overweight women had 3.1 times the odds of anovulatory infertility, and severely underweight women also had increased odds compared to women with a normal BMI (59). Proper diet, healthy exercise and weight loss management have been shown in studies to help treat PCOS, increase fecundability and increase odds of natural conception by facilitating spontaneous ovulation (49). However, a bad "fertility" diet from one study included higher intake of trans fats and animal protein, lower intake of high-fiber, plant-based iron and multivitamin use showed to increase the risk of anovulatory infertility (35,36). While vigorous exercise can improve fertility among overweight and obese women, women with normal BMI that exercise vigorously everyday including cycling, running and swimming have increased odds of anovulatory infertility (43,60). Though the research examining infertility and alcohol consumption is inconsistent and needs to be further elucidated, heavy alcohol use has been shown to increase estrogen levels through a decrease in FSH secretion which can then lead to ovulatory dysfunction (9,61). Caffeine's association with infertility is also not well-defined, with some studies showing an increased risk for higher levels of consumption and others showing no association. The biological mechanisms are unclear; however, some studies theorized that caffeine may affect ovulation through disruptions in glucose metabolism and insulin production (9,62,63).

Structural Dysfunction

Structural or anatomical dysfunction within the fallopian tubes, uterus and cervix can all obstruct the sperm from joining the egg, block transport of zygote to uterus and impair implantation of embryo into the endometrium. Tubal factor infertility accounts for the majority of the structural abnormalities, affecting approximately 30% of female infertility cases (5,49). Some of the main causes of tubal disease include sexually transmitted diseases (STD) such as untreated gonorrhea and chlamydia; which can lead to pelvic inflammatory disease (PID), prior tubal or pelvic surgery, septic abortion and appendicitis (5,49). One large study showed that women with tubal factor infertility had higher odds of chlamydia trachomatis antibodies compared to infertile women who did not have tubal blockage (46). Another study showed that a recurrent PID infection was significantly associated with twice the odds of tubal infertility (45). In most developed countries such as North America and Europe, infections affecting tubal infertility mostly include chlamydia and PID, however, in developing countries like India and Nepal, tuberculosis is the more common source of infection. Previous history of a ruptured appendix was also significantly associated with tubal factor infertility, but not appendicitis (64).

Congenital malformations, cervical trauma, chronic infections of the cervix and surgical treatments such as loop electrosurgical excision (LEEP) procedure used for human papillomavirus (HPV) associated cervical lesions can lead to cervical factor infertility (5,65). Most of these factors impair fertility by affecting the quality and quantity of cervical mucus (65). The cervical mucus helps to protect the sperm within the

acidic environment of the vagina and improve motility to cervix (5). One meta-analysis reviewed six clinical studies and found a significant association between high risk-HPV infection and female infertility (66).

Uterine Abnormalities

Uterine factor infertility is the term used for abnormalities of the uterus that can lead to infertility. It includes uterine fibroids, uterine polyps, uterine scarring or adhesions and endometriosis (5,49). Uterine polyps are formed within the endometrial lining and can impair the function of the uterus. They typically are noncancerous and can regress over time. Fibroids, on the other hand, are made up of thick muscle tissue and are more common, occurring in up to 60% of women. Higher rates of uterine fibroids are found among older women and women of African American race (43). While most women with fibroids can get pregnant, uterine fibroids contribute to 5 to 10% of all female infertility cases (5). They can be found at various locations: within the uterus, endometrium and around the cervix (67). The fibroids that grow within the uterine cavity have the most potential to cause infertility by either obstructing the fallopian tubes, impairing blood supply, altering the shape of the uterus or altering position of the cervix (5,67,68). Pelvic adhesions also known as scarring are bands of fibrous (scar) tissue that form within the uterus from a previous surgery, an injury or an infection (5). They can physically block sperm from traveling to fertilize an egg, as well as, impair blood supply to the uterus consequently affecting implantation (5,67).

Endometriosis is a disease characterized by the growth of uterine tissue outside the uterus. Approximately 10 to 15% of reproductive women are diagnosed with

endometriosis, however, the prevalence rates among infertile women are much higher ranging from 25% to 50% (5,69). The biological mechanisms behind this association are still unknown and controversial but there are proposed theories. They include infertility through pelvic adhesions or scarring which can impair egg release, excessive fluid within the peritoneum which can disrupt the functions of the sperm, eggs and tubes, and lastly, chemical changes within the endometriosis which can lead to implantation failure (5,69).

Treatment Options

Evaluation of female infertility starts with extensive medical and reproductive history and physical examination, along with vital laboratory and imaging tests (6). Treatment options depend on the evaluation and the type of infertility diagnosed. Treating the underlying cause of infertility is the first step. This can be as simple as lifestyle changes such as stopping tobacco use, maintaining a healthy BMI and proper diet, reducing stress, caffeine and alcohol intake and increasing frequency and proper timing of intercourse (5,6). Women with anovulatory infertility can use medications to stimulate ovulation known as ovulation induction (OI). Medications include letrozole, clomiphene or clomiphene citrate, human chorionic gonatropin (hCG) and gonadotropins such as FSH (5,6,49). Infertile women with thyroid disease can also take medications to regulate their hormones and increases chances of conception. Surgery is more effective for tubal and uterine factor infertility. Blockages can be removed from fallopian tubes and tubes can be repaired, however, there is a low success rate and high risk of ectopic pregnancies (5). Uterine scarring from endometriosis, polyps and fibroids can also be surgically removed to increase the odds of pregnancy.

Intrauterine insemination (IUI) is a technique involving the preparation of a semen specimen which is then inserted into the uterus via a catheter (4,5). It is commonly used among uterine, cervical or male factor infertility. IUIs can also be used in combination with OI to increase the odds of conception (5,65). The next line of treatment is ART which per the 2017 International Glossary of Infertility and Fertility Care, does not include OIs and IUIs that involves the handling of only one gamete (4). The glossary uses the term MAR to define any and all technologies to treat different types of infertility diagnoses, including OIs IUI and ART (4). If ART using one's own eggs or a partner's sperm is unsuccessful, couples can use donors, as well as embryo donors, surrogates and gestational carriers (70).

Assisted Reproductive Technology (ART)

Assisted Reproductive Technology is a medical treatment for infertility that handles both sperm and egg for conception and do not include treatments such as IUI or OI (4). ART include but are not limited to include in-vitro fertilization (IVF), IVF with embryo transfer and IVF with intracytoplasmic sperm injection (IVF-ICSI) (7,71). IVF is the main form of ART and involves retrieving a woman's eggs usually after OI, fertilizing the eggs with a semen specimen in a laboratory, then implanting the most optimal embryo(s) into the woman's uterus or a gestational carrier (71). For couples with a diagnosis of male factor infertility, ICSI is incorporated and involves a single sperm being injected into the egg for fertilization (72). In the U.S. approximately 60% of IVF procedures use ICSI (72).

ART success rates can depend on patients' characteristics such age, infertility diagnosis, parity/gravidity, previous miscarriages, lifestyle choices and others factors (7). These include the number and quality of embryos transferred, the type and numbers of ART cycles and use of ICSI also affect success rates. There are different ways to measure ART success rates. The most commonly used rate reported is the percentage of IVF cycles or embryo transfers that resulted in a live birth (7). IVF success rate can also be measured as the percentages of IVF cycles or embryo transfers that resulted in a pregnancy (72).

Epidemiology of MAR

One global report done by the ICMART used a retrospective cross-sectional study to investigate trends over time during 2011. The study involved both women and men undergoing ART from 65 different countries, which is around two thirds of global ART activity (73). Data was imputed for clinics that did not report ART information. There was a reported total of 1,643,912 cycles that resulted in more than 394,662 live born infants (73). There was also an increase in the proportion of women aged 40 years and older undergoing ART from 23.2% in 2010 to 24.0% in 2011 and a slight drop in ICSI percentages from 67.4% in 2010 to 66.5% in 2011 (73). From 2010 to 2011, results also showed an increase in cumulative delivery rates per cycle, a decrease in perinatal mortality rate after fresh IVF/ISCI cycle and even lower drop among perinatal mortality rates after frozen ET (FET) (73).

In the U.S. the CDC released the 2017 Fertility Clinic Success Rates Report and estimated that there were 284,385 ART cycles which had increased from 231,936 in

2015. This resulted in 68,908 live births in 2017, an increase from 60,778 live births in 2015 (7). In the U.S., 1.7% of all infants born in 2017 were conceived through ART services (7). One article published estimates of trends for infertility services, using the NSFG data from 1982 to 2010. They found 12% (7.3 million) of 15 to 44-year-old women reported ever using infertility services from 2006 through 2010 (74). They also found that women who ever used infertility services were older, nulliparous, of non-Hispanic white race, had infertility issues, and were more likely to have higher income and education (74). Additionally, a recent survey in 2019 using NSFG data, showed an increase in ever use of infertility services with 12.7% for 15 to 49-year-old women (24).

SECTION B - Adverse Pregnancy Outcomes

Early pregnancy loss (EPL) of an intrauterine pregnancy prior to 20 weeks' gestation, without the use of elective medical or surgical means to terminate the pregnancy is known as a spontaneous abortion (SA) or miscarriage (10,75). However, the 2017 International Glossary of Infertility and Fertility Care uses 22 weeks of gestational age to define SA (4). Previous nomenclature also included blighted ovum and missed abortion. Spontaneous abortions can be subdivided into several categories such as incomplete abortion, complete abortion, threatened abortion, inevitable abortion, missed abortion, septic abortion and recurrent spontaneous abortion (10). These inconsistencies in the definition and length of gestation make it difficult to compare studies. Early pregnancy loss is a public health burden in that it can be a traumatic experience for many

and is shown to have adverse physical and mental effects including anxiety, depression and grief (10).

Epidemiology of Early Pregnancy Loss

The incidence of EPL is difficult to calculate as many go unrecognized. Most women are unaware of the onset of pregnancy and may misinterpret a SA for a late or heavy menstrual cycle (10,75). Early pregnancy losses can occur either between post-implantation and pre-clinical detection or post medical recognition (75). After four to six weeks of gestational age, the rate of EPL ranges from 10% to 15% and decreases as gestational age increases (76). An early study found that approximately 20% of all clinically detected pregnancies resulted in an early loss (77). However, that number increased to 31%, when women with a biomarker measurement (e.g. hCG) were followed up (77). A recent study in the U.S. analyzed trends in the risk of EPL and found a rate of 13.5% during 1990 through 2000, with a 1% increase per year adjusted for maternal and pregnancy factors (78). Spontaneous abortion rates among ART-assisted conceptions were significantly higher with rates ranging from 8% to 30% compared to the general population (8). One retrospective cohort study found a significant 20% increased risk of SA among ART-assisted pregnancies compared to natural conceptions (8). However, these number may be biased in that ART procedures allow for earlier and more effective detection of losses, as well as the characteristics of infertile women may also predispose their risk.

Predictors of Spontaneous Abortion

There are many common risk factors that can predispose women to have an increased risk of SA including maternal age, prior pregnancy loss, certain medical conditions, substance and specific medication use, some lifestyle factors and environmental exposures (10,79). Etiologies of SAs can be grouped into endocrine dysfunction, immunological causes, infectious diseases, abnormalities in implantation, structural dysfunction of the uterus and most commonly, genetic factors (75,79). The causes and risk factors are discussed below, along with their biological mechanisms.

Up to 90% of EPLs are caused by both numerical and structural chromosomal abnormalities (10). Aneuploidies are numerical chromosomal mutations that result in less or more than the usual number of chromosomes such as in Turner's syndrome. Polyploidy, on the other hand, is defined as having an extra complete set of chromosomes (10,79). The risk of aneuploidies increases with advancing age, along with the risk of SA. One study showed that the risk of EPL varied based on maternal age, for women under 25 years the risk of EPL was around 8.9%, and up to almost 75% among women older than 45 years of age (80). Another more recent study done in Norway, also showed that the risk was lowest for women under 25 years but gradually started increasing after the age of 30, with 53% risk of SA among women older than 45 years (81).

A prior miscarriage is an important risk factor for subsequent losses, after controlling for maternal age (10). The previously mentioned study also examined the risk of SA recurrence and found that after adjusting for age, the odds of having another SA was 60% higher after one prior SA, more than twice the odds after 2 previous SAs and almost four times the odds after 3 prior SAs (81). Medical conditions specifically viral and bacterial infections account for over 15% of all cases and include cytomegalovirus

(CMV), human parvovirus B19, herpes simplex virus, varicella zoster virus and many other infections (10,79). One study showed that the odds of the presence of CMV antibodies were 2.5 times higher among SA compared to normal live births (82). Other maternal diseases such as thyroid dysfunction, PCOS, endometriosis, diabetes, celiac disease and other autoimmune diseases have also been associated with SA (75,79,83). Characteristics of PCOS such as obesity, high levels of LH and insulin resistance have also shown to increase the risk of SA, independent of PCOS (75,83).

One way that immunological factors can cause miscarriage involves the foreign paternal genes inherited within the zygote (75,79). Certain immunological characteristics of the maternal environmental helps to prevent attack or rejection of the inherited paternal genes, thus promoting successful fertilization, implantation and development (75,83). Lack of expression of antiphospholipid antibodies (APA), human leukocyte antigens (HLA), antisperm antibodies (ASA), leukemia inhibitory factor (LIF), integrins, endometrial adhesion factors, cytokines and uterine natural killer cells have all been associated with both infertility and SA (79).

Uterine abnormalities are not as common but include uterine or pelvic adhesions, uterine polyps and uterine fibroids (10). Congenital uterine malformations, such as a septate uterus, have shown to significantly increase the risk of first trimester miscarriage by almost 3-fold (84). Certain medications such as misoprostol (Cytotec), retinoids, methotrexate and nonsteroidal anti-inflammatory drugs including ibuprofen have also been found to increase EPL risk (10). The severity of the risk depends on the drug, the exposure window and the dose or concentration. Environmental exposures such as arsenic, lead, carbon disulfide, heavy metals and organic solvents may also increase the

risk of EPL through apoptosis, impairment of tissue development, or disruptions within cell division and other processes (75,79). Lastly, lifestyle factors that may increase the risk of EPLs include moderate alcohol consumption, heavy caffeine use, substance abuse with methamphetamines, marijuana and cocaine and cigarette smoking, through a dose-response effect (10,75).

SECTION C - Smoking and Secondhand Smoke Exposure

The WHO reported in May 2017 that over 6 million people die yearly as a direct result of tobacco use and over 890,000 people solely from exposure to secondhand smoke (85). Secondhand tobacco smoke (SHS) also known as environmental tobacco smoke (ETS) is a combination of the smoke produced from the burning of tobacco products such as cigarettes, cigars, pipes or bidis and the smoke exhaled by the person smoking a tobacco product (85). There is no safe level of SHS exposure and almost everyone is at risk (85). Globally the prevalence of smoking and is decreasing; however, the absolute number of smokers remains significantly high due to accelerated population growth and an increase in the amount and variety of tobacco products on the market (85).

Epidemiology of Tobacco Use

In 2012, a worldwide study reported that approximately 1 billion men and 250 million women smoked every day, and the prevalence of smoking among women alone was estimated to increase from 12% in 2010 to 20% in 2025 (86). The CDC reported in 2018 that the prevalence of current cigarette smoking among adults aged 18 years and

older in the U.S. is 13.7%, equivalent to 34.2 million adults (87). In the same year, the U.S. reported that 12% of adult females in the U.S. were current smokers (87). A study using the Pregnancy Risk Assessment and Monitoring Survey (PRAMS) data from 27 different sites estimated the prevalence of smoking before pregnancy in 2010. They showed approximately 23% of women reported smoking 3 months before pregnancy, with trends from 2000 through 2010 unchanged for 20% of the women (88). Another study examined the prevalence of self-reported smoking along with intensity of smoking three months before pregnancy in 2016. They found that the overall rate of low-intensity smoking (< 10 cigs/day) was 9.4% and 6.3% for high-intensity smoking (≥ 10 cigs/day) (89).

While there have been significant decreases in current cigarette smoking rates, the level of SHS exposure over the past 30 years remains high. The CDC reported over 58 million non-smokers were exposed to SHS in the U.S. during 2011 to 2012 (90). The total U.S. economic burden directly attributable to smoking for both direct medical care for adults and lost productivity due to deaths and SHS exposure was over $300 billion a year (91). In 2018, the CDC reported from the Behavior Risk Factor Surveillance System (BRFSS) that the percentage of current cigarette use among adults in Kentucky was 23.4%, compared to the national percentage of 16.1% (92). They also reported that the percentage of women aged 18 to 44 years, who reported smoking at least 100 cigarettes in their lifetime and currently smoke every or some days was 23.3% (92).

Types of Tobacco Smoke Exposures

There are several ways to be exposed to tobacco smoke. Mainstream smoke is the smoke that is inhaled then exhaled by a smoker, while sidestream smoke is the smoke emitted into the surrounding environment directly from the burning end of the cigarette, pipe, or cigar (93). The differences in the chemical compositions between mainstream smoke and sidestream smoke depends on the burning conditions. During inhalation, the cigarette burns at a higher temperature allowing for complete combustion in mainstream smoke (93). However, the incomplete combustion from sidestream smoke produces undiluted chemicals that are more concentrated with harmful and carcinogenic chemicals compared to mainstream smoke (93). SHS is a mixture of two forms of smoke from burning tobacco: the exhaled mainstream smoke and sidestream smoke (93). People are also exposed to SHS in their homes, workplaces, public spaces and cars.

Recently, thirdhand smoke has been recognized as another type of tobacco smoke exposure and is the result of residual tobacco smoke chemicals accumulating and attaching to hair, surfaces, clothing, furniture and dusts in the environment (94). These toxins remain settled for months long after the SHS is cleared and over time can become increasingly more dangerous (94,95). One study in 2014 examined the effects of thirdhand smoke using animal models under similar conditions that simulate human exposures. They found that the mice exposed to thirdhand smoke showed changes in multiple organ systems, specifically the liver, lung and skin (95). They found higher lipid levels and non-alcoholic fatty liver disease within the livers of thirdhand smoke exposed mice. In the lung, there were an excess of collagen and inflammatory cytokines and lastly, poor healing of wounded skin on mice exposed to thirdhand smoke (95).

Tobacco Smoke Composition

There are over 7,000 chemicals in tobacco smoke, of which at least 250 are known to be harmful and over 70 that are carcinogenic (96). Some known human carcinogens in SHS are benzene, 2-napthylamine, 4-aminobiphenyl, nickel and polonium-201. Probable human carcinogens in SHS include formaldehyde, hydrazine, 1,3-butadiene, benzo[a]pyrene and cadmium. Some of the toxic substances include carbon monoxide, acrolein, ammonia and nitrogen gases (93,97). Smoking tobacco can potentially harm every organ within the human body and there are a plethora of studies associating tobacco smoke, both active smoking and SHS exposure to a multitude of diseases and illnesses. Some health effects of tobacco smoke include cardiovascular disease such as coronary disease and stroke; respiratory disease such as chronic obstructive pulmonary disease and asthma; several types of cancers such as lung, bladder, cervix and liver; and infertility and adverse pregnancy outcomes (11).

Measures of Tobacco Smoke Exposures

There are a few methods to assess and measure tobacco smoke exposure, with varying levels of validity and reliability. The two most commonly used methods are self-report and biomarkers (97). Self-reported exposures can easily be assessed and measured through questionnaires or interviews. Questionnaires are the most convenient and inexpensive method especially for larger observational studies. Unfortunately, there are limitations with self-reported data such as lack of standardized measurements, misclassifications, recall bias and under-reporting (98). However, meta-analyses have showed that self-reported smoking measures can be validated with biochemical

measurements (99). Some examples of questions asked to assess smoking, for both active and passive smoking include “Do you currently smoke tobacco on a daily basis, less than daily, or not at all?”, “Have you smoked tobacco in the past?”, “How many cigarettes or packs (or list of other tobacco products) do you smoke a day/week?”, “Have you ever smoked more than 100 cigarettes?”, “How often does anyone smoke inside you home?” and “Did anyone smoke at your work during the past 30 days?” (100).

A more objective and reliable measurement of tobacco smoke exposure is the use of biomarkers. The two most widely used are nicotine and cotinine (101). Nicotine is the primary addictive and major component of tobacco smoke. Its half-life is between 2 and 3 hours within the blood and it is then later excreted through urine (101). However, only 5 to 10 percent of nicotine is excreted in the urine and among the remaining, approximately 80 percent is metabolized to cotinine in the liver (101). The limitations to using nicotine include its short half-life, which limits it from assessing long-term exposures, as well as, the assay used to measure nicotine is costly and must be highly specific due to low levels present (101). Cotinine is a metabolite of nicotine and is the preferred biomarker for tobacco exposure (102). Its half-life ranges from 15 to 20 hours and depend on the type of specimen it is being measured from (102). Cotinine can be measure from blood, urine, saliva and hair follicles. Published cutoff values by exposure levels are different for plasma, urine and saliva. For urinary cotinine, levels less than 10 ng/mL to indicate unexposed non-smoker, 10-200 ng/mL for a passive smoker and above 200 ng/mL indicates an active smoker (103). Another example differentiated active smokers with cotinine levels >14ng/mL, SHS exposed from 0.5-13.9 ng/mL and nonsmokers a <0.5ng/mL (104). One recent study used urinary cotinine levels >30ng/mL

to identify current smoking with 94% sensitivity and 98% specificity (105). There are limitations using biomarkers such as differences in uptake, metabolism and excretion rates, which can increase inter-subject variation. It is also very difficult to differentiate SHS exposures because of lower levels of cotinine.

Zenzes and associates examined cotinine levels in follicular fluid among infertile women by smoking status (106). They enrolled 111 infertile women from a hospital IVF program and classified them into active, passive and non-smokers, using self-reported number of cigarettes smoked for both females and their husbands (106). Cotinine was measured in follicular fluid and was strongly correlated in a dose-dependent manner with the number of cigarettes smoked per day for both active and passive smokers. The authors discussed that there were a lot of inter-variation between cases that reported similar number of cigarettes smoked but suggested it may be due to difference in nicotine metabolism, unreliability of self-reported data and time before last cigarette smoked given cotinine's half-life (106). This was one of the first studies to examine cotinine levels in passive smokers. Cotinine was also detected in all active smokers, most of the passive smokers and some nonsmokers, which they deemed as environmentally exposed passive smokers.

Smoking and Biological Mechanisms

Several mechanisms have been proposed to explain how tobacco smoke exposure (particularly before conception) may be harmful for reproductive function. Scientists have used both animal models and clinical data to develop and support these theories.

Folliculogenesis and Steroidogenesis

Follicular fluid is secreted during folliculogenesis and maintains a healthy environment to promote granulosa cell and oocyte development (107). Cigarette smoke is theorized to be associated with folliculogenesis and ovarian steroidogenesis impairment (107,108). Harmful tobacco smoke constituents can build up within the ovaries resulting in a deleterious follicle environment, causing increased oxidative stress, abnormal intercellular crosstalk, meiosis impairment and activation of cell death pathways (107–111). Chemicals found in tobacco smoke such as cadmium and benzo(A)pyrene (BaP) can act as endocrine disrupting chemicals and lead to impairment of production of estradiol and progesterone (107,108). Cigarette smoke components such as nicotine and cotinine can also inhibit the expansion of the cumulus-oocyte complex (COC) (107,108). The structural and functional processes involved in the expansion of COC is important in oocyte maturation, fertilization and embryo development (107,108).

Oxidative stress is the term used to characterize uncontrolled reactive oxygen species (ROS) production, which is a by-product of oxygen metabolism, or an imbalance between free radicals (pro-oxidants) and antioxidants (109). Heavy metals and other harmful substances within tobacco smoke promote oxidative stress, which can lead to harmful effects on cell membranes, DNA and proteins (107,110). DNA damage can lead to aneuploidies which in turn adversely affect fertilization and pregnancy outcomes (109,110). Studies have shown that tobacco smoke contains over 10^{15} ROS per puff (112) and that higher levels of ROS were associated with lower oocyte yield (113). Higher levels of ROS have also been shown to produce immature and poorer quality embryos in women who were not successful with IVF (114). Khan and associates used a mouse

model to show that SHS exposure is associated with adverse fertility outcomes in female mice, which was not prevented by antioxidant use (115). Mai and colleagues examined cigarette smoke extract on ovulation, oocyte morphology and ovarian gene expression using an animal mode. They found cigarette smoke exposure to be associated with poorer quality of oocytes and a shrink size which they believe is due to oxidative stress (116).

Tobacco smoke has been associated with depletion of the follicle pool and earlier menopause either through apoptosis of maturing follicles or autophagy of primordial follicles (107,109). Apoptosis is the term used for programmed cell death and may be the main cause for smoke-induced loss of primordial follicles. Gannon and colleagues showed that mice that were exposed to cigarette smoke twice a day had a reduction in primordial follicle pool as well as, a higher number of autophagosomes in granulosa cells (117). Several studies have also shown accumulation of cotinine and carcinogens within follicular fluid and granulosa cells, which can induce apoptosis and potentially affect ovarian function by directly depleting oocytes (118,119). In 2009, Tuttle and associates tested whether cigarette smoke exposures, representative of human exposure levels would decrease ovarian follicles by apoptosis using mice (120). They found a significant reduction in the number of ovarian follicles via an increase in a pro-survival B-cell lymphoma 2(Bcl 2) marker, which inhibits apoptosis (120). Plante and colleagues studied the impact of smoking on AMH levels and found that active smoking (not former or passive smoking) was associated with lower AMH levels, inferring that tobacco smoke directly affected the antral follicles and not the primordial follicles (121).

Fallopian Tube, Uterine and Placental Effects

Tobacco smoke components have been shown to impair fertility through fallopian tube and uterine effects (108,111). Cigarettes smoke targets the oviduct leading to an increased number of ectopic pregnancies after exposure and with a dose-response effect (108,111). Toxins in cigarette smoke can also cause tubal dysfunction through decreased quality and quantity of ciliary cell activity, abnormal ciliary beat frequency and infundibular smooth muscle contraction, along with lower oocyte retrieval due to excessive adhesions between the COC and tubal lining that is responsible for transport of the COC and embryo along the tubes to the uterus lining (73,108).

A healthy and prepared uterus and uterine lining is necessary for successful embryo implantation. Several studies have shown lower rates of implantation for smokers compared to nonsmokers among ART users (122–124). The mechanisms behind tobacco smoke's effects on the endometrium are still being elucidated. However, several animal studies have shown that chemicals in cigarette smoke (mostly nicotine, cadmium and BaP) can decrease the weight of the uterus, suppress the uterine response to decidualization, decrease mobility of endothelial cells, and inhibit cell proliferation (108). Early placentation involves growth, differentiation, transport and invasion of the trophoblast (108,111). Few studies using human trophoblastic cells have shown that cigarette smoke chemicals--particularly cadmium, which is a known placental toxin, BaP, and nicotine-- can disrupt proliferation, differentiation and migration (108,111). These effects are also directly related to the increased risk of SA.

SECTION D - Epidemiological Studies

Active Smoking and Fertility

There have been many studies investigating whether active current smoking is associated with TTC or fecundability (26, 117–119), infertility (128–130) and conception outcomes for both natural conceptions (131) and MAR (132,133). Findings have been inconsistent, with some studies showing significant associations and some showing no association (134,135). The following section describes past research on active smoking by different types of fertility outcomes. These studies are summarized in Table 1.

Fecundability

In 1997, Curtis and colleagues examined the effects of active smoking and other risk factors on fecundability, using a retrospective cohort study. Couples from the Ontario Farm Family Health study who were planning to conceive, completed detailed questionnaires self-reporting the number of pregnancies, TTC, detailed exposure information and other important covariates (38). The final sample size included 1277 couples that contributed 2607 pregnancies. After adjusting for spouse's smoking, recent oral contraceptive use, and woman's age, women who smoked had significantly reduced fecundability compared to nonsmokers (adjusted fecundability ratio (aFR): 0.90; 95% confidence intervals (CI): 0.82, 0.98). There was also a significant a dose-response effect for the number of cigarettes per day (aFR: 0.74; 95% Cl: 0.59, 0.92 for >20 cigarettes per day) (38). Limitations of the study above include the lack of generalizability given the specific population, the results may be not be generalizable to the general population. Effects may be overestimated, as smokers who are highly fertile may have been excluded, given that they are more likely to have unplanned pregnancies (38). Also, data

was only collected on couples who did conceive, which excluded potentially infertile, sterile or less fecund couples. And because smoking has been shown to be associated with infertility and fecundability, the estimates may be biased towards a null association.

In 2014, Radin and colleagues used a prospective cohort study to assess active smoking on fecundability also using women planning to conceive (126). Current and past smoking history along with important covariates were collected by questionnaire at baseline. Outcome data were self-reported twice a month using follow-up questionnaires. Confirmation of pregnancy by either a home pregnancy test or physician was collected, along with TTC and last menstrual cycle. The final sample size included 3773 women who contributed 15,774 cycles with 2,578 confirmed pregnancies (126). After adjusting for cycle number at risk, age, partner smoking, and passive smoking, women who were current smokers had lower fecundability than never smokers, but the estimate was not significant (aFR:0.89; 95% CI: 0.77, 1.03) (126). The authors also found that women who were regular smokers for ten years and more had a significantly reduced fecundability than never smokers (aFR:0.85; 95% CI: 0.71, 1.00) (126). No significant dose-response effect was established. As detailed as the exposure assessment was, heavy smokers have been shown to under-report their smoking intensity, leading to exposure misclassification which can lead to a downward bias. The authors also mentioned unmeasured confounding and overestimated effects, if the smokers who that were more likely to be lost to follow-up, were also more likely to conceive (126). Similar to the previous study described, excluding unplanned pregnancies can also lead to an upward bias.

A more recent study in 2019 examined the extent to which cigarette smoking affected fecundability using a North American internet-based cohort of pregnancy

planners. Among 5,473 females, active and passive smoking exposures were self-reported at baseline, then followed up bi-monthly to collect current smoking history for the prior month (135). Conception outcomes were also self-reported and collected at each follow-up, along with TTC measured in cycles. Smoking intensity using number of cigarettes smoked per day and duration were analyzed individually and then jointly among female current smokers. The authors used proportional probabilities regression to estimate FRs and 95% CIs, adjusted for important covariates (135).

Results showed minor insignificant reductions in fecundability for current, female smokers (aFR: 0.90; 95% CI: 0.77, 1.07), current occasional smokers (aFR: 0.88; 95% CI: 0.73, 1.06), and former smokers (aFR: 0.89; 95% CI: 0.81, 0.98) (135). Females who smoked ≥10 cigarettes/day for ≥10 years had stronger associations with reduced fecundability, though still not statistically significant (aFR: 0.77; 95% CI: 0.53, 1.10) (82). The results of this study supported past studies that revealed current smoking is associated with reduced fecundability; however, this study also had a few limitations. Firstly, all smoking exposures were self-reported, which are commonly under-reported and can lead to non-differential misclassification and a downward bias. Results also showed low agreement between female and male reporting of smoking intensity which could be further evidence of under-reporting (135). An objective biomarker such as cotinine could have increased the validity of the estimates and reduce bias. The study excluded women who had been trying to conceive for 6 or more months prior to enrollment and restricted the sample to couples planning a pregnancy. It has been theorized that smokers are more likely to have unplanned pregnancies either because of contraceptive use or risky behaviors, so in theory, less fertile smokers would be enrolled,

which would bias the estimate in an upward direction (135). Lastly, the authors stated that results from additional analyses showed female current smokers were more likely to be lost to follow-up, which can lead to an upward bias, if female smokers who did not conceive were disproportionately lost to follow-up.

Infertility

In 1998, Augood and colleagues conducted a systematic review and meta-analysis on active smoking and female infertility. Twelve studies were used after meeting the stringent criteria of the meta-analysis. There were 4 case-control studies and 8 cohort studies: one prospective and seven retrospective (130). The results overall showed a significant 60% increased odds of infertility among current cigarette smokers when compared to non-smokers (OR: 1.60; 95% CI: 1.34, 1.91) (130). This statistically significant result was also seen when the results were stratified by study designs; for case-control studies (OR:2.27; 95% CI: 1.28, 4.02) and cohort studies (OR:1.42; 95% CI: 1.12, 1.91) (130). Regardless of the limitations of this meta-analysis, the overall precision and consistency of effects across the different study designs, outcomes, and sample sizes like past studies, supported the negative effect of smoking on female fertility (130). However, while some of the individual studies within the meta-analysis were adjusted for important confounders, the overall estimates were not.

Laurent and colleagues used a retrospective case-control study to investigate the effect of active smoking on female infertility. Women used in this study were among the controls of a cancer and steroid hormone study (128). They recruited 482 cases and 2231 controls. Cases of primary female infertility were defined as having 24 months of actively

trying to get pregnancy (unprotected intercourse) without pregnancy. And controls were defined as women who did not meet the criteria for a case, and also had documented fertility (128). Exposure data was self-reported during an interview-administered questionnaire. After adjusting for age at infertility/conception, age at first intercourse, education level, race, and history of benign ovarian disease, the odds of primary infertility were 36% higher for women who smoked a pack a day compared to nonsmokers (adjusted odds ratio (aOR):1.36; 95% CI: 1.14, 1.36) (128). However, there were many limitations including the retrospective study design which cannot assess temporality of the effect to outcome. Also, women who were categorized as infertile may over-report exposures because of known associations with infertility and lastly, smoking behaviors can change over time, leading to misclassification and can lead to overestimated effects. The authors also stated that contraceptive use and treatment of infertility were important confounders that were not adjusted for and can ultimately bias the estimates in an upward direction (128).

In 2016, Hyland and others cross-sectionally examined a large prospective cohort to assess tobacco smoke exposure with infertility. All information including tobacco smoke exposures, infertility history and other relevant variables were extracted from questionnaire used in the Women's Health Initiative observational study (129). Among the 88,732 women, active smokers overall had significant increased odds of infertility compared to never smokers (aOR: 1.14; 95% CI: 1.03, 1.26) after adjusting for several confounders (129). However, there were a few limitations to this study. Firstly, cross-sectional studies are a weak design to infer causality, because it does not allow for temporality, especially given that many women change their smoking behaviors.

Exposures and outcome data were self-reported which can be subjected to recall bias or under-reported leading to misclassification and a downward bias. The authors also stated that there maybe be bias from the lack of adjustment for important confounders such as alcohol use, contraceptive use, infertility treatment and male factor infertility (129).

Clinical Outcomes of Medically Assisted Reproduction

With the emergence of MAR, study designs have improved to allow for more efficient methods in assessing exposures on conception outcomes among infertile women and couples. Several meta-analyses and more recent studies have examined the effect of active smoking on MAR outcomes (131,136–138). Feichtinger and colleagues conducted a meta-analysis to examine the relationship between the clinical pregnancy rate of female smokers after the first IVF-ET cycle (136). The authors used seven articles and one of their own studies to calculate pooled odds ratios and 95% CI. Among a total of 2314 first IVF-ET cycles, it was found that almost twice as many IVF-ET treatment cycles were needed for smokers to conceive compared to non-smokers (OR:1.79, 95%CI: 1.24, 2.59) (136). Given the weak methodological designs of the studies used in this meta-analysis, the authors warned that the accuracy of overall effect estimate should be taken with careful consideration.

Waylen and colleagues also conducted a meta-analysis examining the clinical outcomes ART and smoking at the time of treatment. Among the 18 studies used for the clinical pregnancy rate per cycle outcome, there were 1284 smokers and 3959 matched controls (137). The results showed a significant decrease in clinical pregnancy rate per cycle for women smoking at the time of ART treatment compared to women who did not

(OR: 0.56; 95% CI: 0.43, 0.73) (137). Budani and colleagues updated the above meta-analysis in 2016 to include three additional studies and found similar results for the clinical pregnancy rate per cycle (OR: 0.53; 95% CI: 0.41, 0.68) (138). Similar limitations were mentioned for both, including that the results are more likely to be confounded by age or male smoking status and the lack of external validity (137,138). Also, most of the studies included in both meta-analyses only took into account self-reported smoking status by questionnaire, while only a few used an objective marker.

Summary

Although there are inconsistencies across studies, the body of literature as a whole suggests that current smoking may be associated with reduced fecundability, increased odds of infertility, and lower the odds of both natural and MAR conceptions (Table 1). The associations are weaker, however, among the larger studies. Of the seven cohort studies analyzed, three were of prospective designs and of those, two did not find a significant association. There were only two studies that found significant dose-response effects with the number of cigarettes. Also, cross-sectional analyses, case-control or even retrospective study designs are more susceptible to bias which limits inferences on causality. Lastly, almost all of these studies used self-reported smoking exposures which can lead to misclassification and underestimated associations because of under-reporting. Few studies used an objective biomarker to verify smoking status which can help to increase accuracy of the estimates and reduce bias.

Table 1. Studies on Active Smoking Exposures and Fertility

	Study Design	N	ART	Exposure	Outcome	Estimate	95% CI
Baird & Wilcox, 1985 (125)	TTC	678 women	No	Self-reported	Time to conception	0.72[a]	0.59 - 0.87
Hull et al., 2000 (134)	CS of PCS	8515 women	No	Self-reported	Delayed conception	1.23[a]	0.98 - 1.49
Curtis et al., 1997 (38)	RCS	1277 women	No	Self-reported	Fecundability	0.90[b]	0.82 - 0.98
Radin et al., 2014 (126)	PCS	3773 women	No	Self-reported	Fecundability	0.85[b]	0.72 - 1.00
Sapra et al., 2016 (127)	PCS	501 women	No	Self-reported	Fecundability	0.53[b]	0.33 - 0.85
Wesselink et al., 2019 (135)	PCS	2962 women	No	Self-reported	Fecundability	0.90[b]	0.77 - 1.07
Laurent et al., 1992 (128)	CACO	482 cases, 2231 controls	No	Self-reported	Infertility	1.36[a]	1.14 - 1.61
Hyland et al., 2016 (129)	CS of PCS	88 732 women	No	Self-reported	Infertility	1.14[a]	1.03 - 1.26
Van Voorhis et al.,	RCS	499 women	Yes	Self-reported	Ongoing pregnancy rate	0.32[a]	0.13 - 0.79

1996 (132)							
Freour et al,. 2008 (133)	RCS	111 women	Yes	Self-reported	Clinical pregnancy rate	% difference	NA (p value < 0.05)
Hughes & Brennan, 1996 (131)	MA	13 studies	No	Mostly self-reported, few used biomarkers	Natural conception	0.33 to 1.0 *	NA
		7 studies	Yes		Conceptions per cycle	0.57c *	0.42 - 0.78
Augood et al., 1998 (130)	MA	12 studies	No	Self-reported	Infertility	1.60c *	1.34 - 1.91
Feichtinger et al.,1997 (136)	MA	8 studies	Yes	Self-reported	Clinical pregnancy rate	1.79c *	1.24 - 2.59
		9 studies	Yes	Self-reported	Pregnancies per IVF-treated cycles	0.66c *	0.49 - 0.88
Waylen et al., 2009 (137)	MA	18 studies	Yes	Self-reported and biomarkers	Clinical pregnancy per cycle	0.56c *	0.43 - 0.73
Budani et al., 2018 (138)	MA	21 studies	Yes	Self-reported and biomarkers	Clinical pregnancy rate per cycle	0.53c *	0.41 - 0.68

TTC - Time to conception, CS - Cross-sectional, RCS - Retrospective cohort study, PCS - Prospective cohort study, CACO - Case-control, MA - Meta-analysis
a - Adjusted Odds Ratio, b - Adjusted Fecundability Ratio, c - Pooled OR/RR
* - Some individual studies adjusted for confounders

Secondhand Smoke Exposure and Fertility

Research on SHS exposure with fertility outcomes such as TTC or fecundability (127,134), infertility (129) and natural or assisted conception outcomes (122,124) are few and limited with mostly inconclusive or inconsistent results (126,139,140). The following section describes past research on SHS exposures by different types of fertility outcomes. These studies are summarized in Table 2.

Fecundability

In 2000, Hull and colleagues investigated passive smoking on TTC by retrospectively analyzing a prospective cohort study. Using the Avon Longitudinal Study of Pregnancy and Childhood, exposures, outcome and other relevant variables were self-reported by questionnaire (134). Pregnancies were enrolled at 18 weeks of gestation and both unplanned pregnancies and planned pregnancies that did not reach 24 weeks of gestation were excluded. The final sample included 8,515 women who contributed 9,065 pregnancies (134). After adjusting for confounders, passive smoke exposure was found to significantly delay 6-month conception (aOR: 1.17; 95%CI: 1.02, 1.37) compared to no SHS exposure (134). Possible limitations to this study included the lack of generalizability, unmeasured confounders, the study population which was limited to only fecund women and under-reporting of smoking behaviors, which can lead to misclassification and null associations. This study also did not use an objective biomarker to verify current passive or active smoke exposures.

However, as previously described in 2014, Radin and coauthors used a prospective cohort study to examine passive smoking on fecundability among women

planning to get pregnant. Among 2,346 never smokers, passive smoking was associated with reduced fecundability but was not statistically significant (aFR: 0.92; 95% CI: 0.83, 1.03), after adjusting for confounders (126). The authors stated that as of 2014, their study was the largest prospective study to examine passive smoking and fecundability.

Infertility

Also described earlier, Hyland and colleagues assessed lifetime tobacco smoke exposure on infertility using a cross-sectional design. After adjusting for age, race, education, alcohol use, insecticide exposure, oral contraceptive use, BMI, and exercise, never-smoking women with the highest levels of lifetime SHS exposure had significantly increased odds of infertility compared to nonsmoking women with no SHS exposure (aOR:1.18; 95% CI:1.02, 1.35) (129). Some strengths of this study included its large size which increased statistical power, adjustment of several confounders and thorough assessment the impact of lifetime tobacco exposure (129). However, limitations included the cross-sectional study design which limited causality, recall bias as women were all past reproductive age, social-desirability bias, and not accounting for male factor infertility.

Clinical Outcomes of Medically Assisted Reproduction

Neal and associates conducted a retrospective study to measure cigarette smoke effect from mainstream (MS) and sidestream (SS) smoke-exposed women undergoing IVF on fertility outcomes compared to non-smoking (NS) women (124). Smoking status was self-reported and categorized into three categories as stated above. The fertility

outcomes included embryo quality, implantation rates and pregnancy rates. Among 225 women, results showed a significant difference in implantation rate (MS=12.0%, SS=12.6%, and NS=25.0%, $p<0.001$) and pregnancy rate per embryo transfer (MS=19.4%, SS=20.0%, and NS=48.3%, $p<0.001$), but showed no difference in embryo quality between the three smoking groups (124). Misclassification errors and recall bias may have limited the accuracy of the effect, in that women under-reported exposures which can lead to a downward bias. Other limitations include the lack of an objective marker for smoking exposure and residual confounding.

In 2007, Meeker and colleagues conducted two retrospective analyses of a prospective cohort study to analyze SHS exposure on pregnancy outcomes among women undergoing MAR. The first study used a retrospective cohort study to investigate adverse pregnancy outcomes and maternal exposure to SHS (139). Adverse pregnancy outcomes were collected from medical records and included failure of fertilization, failed implantation and SA. Maternal exposure to SHS was self-reported on questionnaires and urinary cotinine levels adjusted for creatinine were measured at the time of ART treatment. After adjusted for confounders, urinary cotinine levels above the median were not associated with failed implantation (aOR: 0.98; 95% CI: 0.70, 1.37)(139). The second study was done to analyze women with childhood SHS exposure from their parents and SA, with self-reported exposure data. Using a larger sample of 1449 non-smoking women, no significant association was also found between any current self-reported SHS exposures and failed implantation (aOR: 1.43; 95% CI: 0.97, 2.09)(140).

In 2011, Benedict and associates carried out a retrospective analysis of a prospective cohort study to assess the association between SHS exposure and

implantation failure among non-smoking women already in IVF treatment (122). The study enrolled 1909 couples with 3270 total IVF cycles. Medical and lifestyle histories were self-reported. SHS exposure status was self-reported and then verified by measuring cotinine in follicular fluid. After adjusting for confounders, the authors found a significant increase in risk of failed implantations among SHS exposed women compared to unexposed women (aOR = 1.52; 95% CI = 1.20, 1.92; adjusted risk ratio (aRR) = 1.17; 95% CI = 1.10, 1.25) (122). They claimed to have the largest study of SHS exposure using cotinine on fertility outcomes among IVF patients. Some strengths of this study included the use of odds and risk ratios; both statistically significant indicating a robust model, adjustment for several potential confounders and use of an objective marker to verify SHS exposure status. However, the generalizability of the study is limited because the study population only included couples in IVF treatment, which is a very specific demographic. The outcome of implantation failure is usually not observed outside MAR population and choosing the best embryos in a laboratory is not an option in the general population (122).

Summary

There are only a few studies examining the association of SHS exposure and conception outcomes and some are summarized in Table 2. However, the available studies are inconsistent and do not have strong associations. Most of the studies were cross-sectional in design rather than the ideal prospective cohort, which would allow for temporality to infer causality. Only one of three cohort studies found significant results, however, that study was relatively smaller compared to the others. Furthermore, three of

the eight studies used a biomarker to measure and verify SHS exposure, the others relied on self-reporting which has been shown to result in null association. Lastly, adjustment of important confounders and the use of biomarkers such as cotinine are necessary to reduce bias in the association of SHS exposure on conception.

Table 2: Studies on Secondhand Smoke Exposures and Fertility

	Study Design	N	ART	Exposure	Outcome	Estimate	95% CI
Hull et al., 2000 (134)	CS	8515 women	No	Self-reported	Delayed conception	1.17[b]	1.02 - 1.37
Radin et al., 2014 (126)	PCS	3773 women	No	Self-reported	Fecundability	0.92[c]	0.82–1.03
Sapra et al., 2016 (127)	PCS	501 women	No	Serum cotinine	Fecundability	0.64[c]	0.41 - 0.98
Wesselink et. al., 2019 (135)	PCS	2962 women	No	Self-reported	Fecundability	0.93[c]	0.70 - 1.25
Hyland et al., 2016 (129)	CS of PCS	88 732 women	No	Self-reported	Infertility	1.18[b]	1.02 - 1.35
Neal et al., 2005 (124)	RCS	225 women	Yes	Self-reported	Implantation rate	Difference in rate	p-value <0.01
Meeker et al., 2007a (139)	CS of PCS	921 women	Yes	Urinary cotinine	Implantation failure	0.98[b]	0.70–1.37

Meeker et al., 2007b (140)	CS of PCS	1449 women	No	Self-reported	Implantation failure	1.43[b]	0.97–2.09
Benedict et al., 2011 (122)	CS of PCS	1909 women	Yes	Follicular fluid cotinine	Implantation failure	1.17[a], 1.52[b]	1.20–1.92, 1.10–1.25

TTC - Time to conception, CS - Cross-sectional, RCS - Retrospective cohort study, PCS - Prospective cohort study, CACO - Case-control, MA - Meta-analysis
a - Adjusted risk ratio
b - Adjusted odds ratio
c - Adjusted Fecundability Ratio
d - Pooled OR/RR
* - Some individual studies adjusted for confounders

Active Smoking on Spontaneous Abortion

Past studies have shown that active smoking affects early pregnancy loss for both natural (141–145) and assisted conception (123,146). However, most of these studies measured tobacco use during pregnancy and only a few took into account exposures before conception or before MAR procedures (144,147). In 1999, Ness and colleagues examined active smoking on SA among pregnant adolescents and women aged 14 to 40 years who visited the emergency department at the University of Pennsylvania Hospital (142). Tobacco use after conception, during the pregnancy was self-reported and verified by urinary cotinine. Women with urinary cotinine levels great than 500 ng/mL were defined as heavy smokers. Four hundred pregnant subjects had a SA either within 3 days of enrollment or during follow-up and 570 women (controls) remained pregnant past 22 weeks of gestation (142). Results showed that heavy smoking was independently associated with an 80% increased risk in SA (aOR: 1.8 (95%CI: 1.3, 2.6) (142). However, the authors discussed several limitations including residual confounding from

unmeasured factors, recall and misclassification bias and urinary cotinine's half-life which only measured recent tobacco use.(142).

One study that examined tobacco exposures prior to pregnancy on SA was conducted in 2006 by Nielsen and associates. Using a nested case-control study from a population-based cohort comprising of 20 to 29-year-old women established from 1991 to 1993. The authors recruited 343 cases of SA and 1578 women who had live births (144). Detailed pre-conception tobacco smoke exposures were self-reported during an interview-administered questionnaire at enrollment. After adjusting for age, marital status, previous SA, use of oral contraceptives, use of intrauterine devices, current smoking status, cigarette smoked per day, duration of smoking, and time since quit smoking; pre-conception smoking was significantly associated with SA (aOR: 1.64; 95% CI: 1.07, 2.50) (144). A significant dose-response effect was also found among the number of cigarettes smoked per day and SA (aOR: 1.20; 95% CI: 1.04, 1.39). However, no association was for duration of smoking (144). While this study warranted the need for further investigation of pre-pregnancy smoking habits on SA and had many strengths, there were a few limitations.

The study suggested that pre-conception smoking may be a proxy for smoking during pregnancy. It has been shown that heavy pre-conception smokers may have a harder time quitting during pregnancy, which can be relevant in predicting smoking during pregnancy (144). However, because data on smoking during pregnancy was not collected, this assumption was not tested which limited the study effect estimates. As it was unclear whether the effect estimates were truly a result of pre-conception smoking or if pre-conception smoking acted as a proxy for smoking during pregnancy (144). Other

limitations included recall bias, unmeasured factors such as SHS exposure from spouse or work and the lack of differentiation between early and late SA.

Spontaneous Abortion after Medically Assisted Reproduction

The relationship between active smoking and clinical outcomes within MAR helps to add and improve the research among the specific population, by allowing for earlier and efficient detection of pregnancy losses. One study done in 2005 assessed the effects on smoking with the IVF success rates among infertile couples. The authors retrospectively analyzed data from a large prospective cohort study and used 8457 eligible women with first IVF cycles (146). Exposures, outcome and relevant information were either extracted from the medical record or self-reported through a mailed questionnaire. Smokers were defined as having smoked more than one cigarette a day, for one year or more, at the time of oocyte retrieval. After adjusting for age, BMI, different infertility diagnoses and duration of subfertility, current smoking was found to significantly lower of odds of live birth per IVF cycle compared to nonsmokers (aOR: 0.72; 95% CI: 0.61, 0.84) (146). The authors conducted non-responder analysis which showed that women with live births were more likely to respond and participate than women with unfavorable outcomes. This may have caused an overestimation of IVF-assisted birth rates which can result in biased estimates in upward direction, if non-response was associated with the smoking exposure (146).

In 2019, Rockhill and colleagues used a retrospective cohort in the U.S. to estimate the proportion of ART cycles with self-reported smoking, and its association with ART related outcomes (147). Women who self-reported any smoking 3 months

before the treatment cycle were defined as smokers. Using national data from 2009 through 2013, outcome data was collected for every ART cycle and included cancellations of ART cycles for all cycles, cycles before egg retrieval and cycles before ET/FET (147). Pregnancy outcomes such as SA, stillbirth and live birth among cycles with at least one ET were also collected and examined. Logistic regression was used to estimate ORs and 95% CIs, taking into account clustering by state, clinic and patient for smoking and intensity of smoking with the previously mentioned outcomes (147).

Results showed that smoking was reported in over 12,000 (1.9%) ART cycles and that smokers were more likely to be younger, multiparous, of non-Hispanic White race and be diagnosed with tubal and male factor infertility (147). Smokers had lowered odds of implantation, IUP and live birth, along with an increase in the odds of SA, however, all estimates were not statistically significant (147). While this study incorporated a large, nationally representative sample and found results consistent with past studies, limitations included recall bias and misclassification, as exposure status was not verified with a biomarker. The authors also stated that women undergoing ART treatment tend to under-report their smoking habits, which can lead to exposure misclassification and consequently, bias estimates toward the null. The study did not address partners' smoking history or SHS exposure, which have shown to be important risk factors.

Summary

The 2020 U.S. Surgeon General report stated that there is suggestive but insufficient evidence of a causal relationship between maternal smoking and SA (12). However, a number of studies examining the association were not used in the report. As

shown in Table 3, there is a lack of prospective cohort studies in this area. The majority are cross sectional, case-control or retrospective designs which are susceptible to misclassification, and cannot assess temporality, which threaten inferential causality. There is also a paucity of studies examining pre-conception smoking and among past smokers. Exploring smoking at different time periods such as before and during pregnancy, can help further elucidate potential mechanisms.

Table 3: Studies on Active Smoking and Spontaneous Abortion

	Study Design	N	ART	Exposure	Outcome	Estimate	95% CI
Armstrong et al., 1992 (141)	CS	~56000 women	No	Self-reported	SA	1.68[a]	1.57 - 1.79
Ness et al., 1999 (142)	CACO	400 cases, 570 controls	No	Urinary Cotinine	SA	1.80[a]	1.3 - 2.6
Mishra et al., 2000 (143)	CS	2617 women	No	Self-reported	SA	2.00[a]	1.50 - 2.80
Winter et al., 2002 (123)	RCS	1196 pregnancies	Yes	Self-reported	EPL	2.00[a]	1.27 - 3.15
Linsten et al., 2005 (146)	CS of PCS	8457 women w/ 1st IVF cycle	Yes	Self-reported	Live birth rate after IVF	0.72[a]	0.61 - 0.84
Nielsen et al., 2006 (144)	Nested CACO	343 cases, 1578 controls	No	Self-reported (pre-conception)	SA	1.20[a]	1.04 - 1.39
Hyland et al., 2015	CS	77 805 women	No	Self-reported	SA	1.16[a]	1.08 - 1.26

(145)							
Rockhill et al., 2019 (147)	RCS	175885 ART cycles	Yes	Self-reported (pre-ART)	SA	1.05[a]	0.96 - 1.15
Hughes and Brennan, 1996 (131)	MA	7 studies	No	Mostly self-reported, few biomarkers	SA	0.83 - 1.8*	NA
Waylen et al., 2009 (137)	MA	4 studies (100 to 6903 cycles)	Yes	Self-reported and biomarkers	Live birth per cycle	0.54[b] *	0.30 - 0.99
		7 studies (211 smokers, 3959 matched controls)	Yes	Self-reported and biomarkers	SA	2.65[b] *	1.33 - 5.30
Pineles et al., 2014 (148)	MA	50 studies	Both	Self-reported and biomarkers	SA	1.23[b] *	1.16 - 1.30
Budani et al., 2018 (138)	MA	7 studies (3407 smokers, 738 nonsmokers)	No	Self-reported and biomarkers	Live birth rate per cycle	0.59[b] *	0.44 - 0.79
		8 studies (226 smokers, 1796 nonsmokers)	Yes	Self-reported and biomarker	SA rate per clinical pregnancy	2.22[b] *	1.10 - 4.48

CS - Cross-sectional, RCS - Retrospective cohort study, PCS - Prospective cohort study, CACO - Case-control, MA - Meta-analysis

a - Adjusted odds ratio

b - Pooled OR/RR

* - Some individual studies adjusted for confounders

Secondhand Smoke Exposure and Spontaneous Abortion

Several individual studies have examined SHS exposure and SA (122,145,149–151), however, studies are limited and results are inconsistent (139,140,152). Hyland and colleagues used a large cross-sectional study to examine the effect of lifetime SHS exposure among 40,850 never-smoking women from the Women's Health Initiative observational study. Exposures, outcome and relevant covariate data were self-reported via questionnaire (145). Nonsmoking women with the highest levels of lifetime SHS exposure, including childhood SHS exposure greater than 10 years; adult home SHS exposure greater than 20 years and adult work SHS exposure for more than 10 years, had a significantly increased odds of SA compared to nonsmoking women with no SHS exposure (aOR: 1.17; 95% CI: 1.05, 1.30) after adjusting for age, BMI, gravidity, oral contraceptive use, race, education and alcohol use (145). A significant dose-response effect was also established among SHS exposure and SA (p value = 0.01) (145). Limitations included the cross-sectional study design which is a weaker design to infer causation and under-reporting of tobacco smoke exposures which can lead to misclassification and a downward bias. The authors stated that home pregnancy tests were not readily available which limited the outcome to be recognized by the patient or by physician and could have also driven estimates toward the null (145).

A large clinical prospective cohort study was done in 1991 by Ahlborg and Bodin, examined passive smoke exposure during pregnancy on pregnancy outcomes. The study enrolled 4,787 women attending prenatal care centers in Sweden (149). The authors defined pregnancy outcome as either SA or stillbirth and relied on self-reported exposure data. The only significant results found were among first-trimester intrauterine deaths,

which showed an increased risk for passively exposed women at work (aRR:2.16; 95% CI:1.23, 3.81) (149). They also showed that active smoking was associated with intrauterine deaths, but not passive smoke exposure in the home. While this was a large prospective study, results may be biased because of self-reported data and lack of adjustment of important confounders. The authors also stated that more quantitative measures of exposure at different time points were another limitation to their study.

In 1992, Windham and colleagues used a large case-control study to examine maternal SHS exposure during pregnancy and SA (150). Cases were defined as women who had a SA recorded at local hospitals and exposure information were self-reported via a telephone interview. Results showed that pregnant women exposed to SHS for an hour or more a day had significantly increased of odds of SA (aOR:1.5; 95% CI: 1.2, 1.9) compared to women who reported no SHS exposure (150). However, the same authors conducted a similar study using a large prospective cohort of 5000 women in 1999 and found no significant results (aOR1.01; 95% CI: 0.80, 1.27) between maternal SHS exposure and SA (152). This latter study differentiated SHS exposure from home, work or either compared to the previous study in 1992. While the prospective cohort was a larger sample, the authors reported that similar limitations such as reporting bias, misclassification, unidentified confounders and lack of more objective, quantitative exposure measure such as cotinine to accurately measure SHS exposure (150,152). These contradicting results further add to the inconsistencies among estimates from studies analyzing SHS exposure and SA.

In 2006, George and colleagues used a population-based case-control study to examine the relationship of SA with SHS exposure using plasma cotinine concentrations

(151). The authors claimed to have conducted the first study assessing SHS exposure using plasma cotinine levels and SA. Cases were defined as having a SA at 6 or 12 weeks of gestation and exposure information was self-reported weekly before and after conception (151). Plasma cotinine was measured from blood samples taken at time of miscarriage. Active smokers were defined as having cotinine levels greater than 15 ng/mL and SHS exposure between 0.1 and 15 ng/mL (151). Nonsmokers had plasma cotinine levels less than 0.1 ng/mL. The results after adjusting for relevant confounders, showed significant increases in the odds of SA for SHS exposed women (aOR:1.67; 95% CI: 1.17, 2.38) compared to nonsmokers (151). Some possible limitations included the study design, using a case-control study does not allow for temporality to be established, and the authors did not examine dose-response relationships which is also important in assessing causality.

Meta-analyses conducted have also shown contradicting results (148,153,154). In 2011, Leonardi-Bee and others conducted a systematic review and meta-analysis of 19 studies to assess the risk of adverse fetal outcomes among nonsmoking pregnant women with SHS exposure (154). They looked at several outcomes including SA which was defined as death before 20 weeks' gestation and stillbirth, defined as death between 20 weeks' gestation and birth. SHS exposures were assessed either through self-report or a biochemical measure. The authors did not find a significant association among SHS exposure and SA from 6 studies (pooled OR: 1.17; 95% CI: 0.88, 1.54) (154). The authors conducted sensitivity analyses with similar findings and reported low heterogeneity between studies. Also, most of the studies relied on self-ported exposures and pooled ORs were not adjusted for important confounders. Another significant

limitation of all the previously mentioned studies is a lack of assessment of infertility within the association of SHS exposure and SA. As stated before, there is a significant increased prevalence of SA among the in infertile population and how it may modify the association is essential in accurately estimating an unbiased effect.

Spontaneous Abortion after Medically Assisted Reproduction

Conception and pregnancy outcome studies can be designed to better explore risk factors since the emergence of MAR. As previously described, Meeker and colleagues analyzed adverse pregnancy outcomes and maternal exposure to SHS among women undergoing ART treatment (139). Maternal exposure to SHS was self-reported on questionnaires and urinary cotinine levels adjusted for creatinine were measured at the time of ART treatment. Among all non-smokers, the median cotinine concentration adjusted for creatinine was 57.1 ng/mL. After adjusting for confounders, no significant association was found between urinary cotinine and SA (aOR: 0.51; 95% CI: 0.21, 1.24) (139). The authors then used a larger population of 1449 women to analyze women with childhood SHS exposure from their parents and SA, using self-reported exposure data. The authors also found no association with any current SHS exposure and SA (aOR: 0.80; 95% CI: 0.30, 2.14), as well as, no significant dose-response effects (140). However, childhood SHS exposure was found to have significantly higher odds of SA compared to women with no childhood SHS exposure (140). Limitations for the latter study included the lack of external validity and the high levels of discordance between urinary cotinine and self-reported SHS exposures. The authors stated that other places of exposures besides at home and at work were not assessed and given cotinine's half-life,

one measurement can lead to misclassification (139,140). They also discussed the dose-response fallacy where the exposure threshold is lower than expected for the lowest exposed group, leading to underestimated effects (140).

As described earlier, Benedict and associates also examined SHS exposure on IVF success rates with a retrospective analysis of a prospective cohort study. Cotinine in follicular fluid was measured at the time of egg retrieval per cycle. Women with levels greater than 1.11 ng/mL were defined as SHS exposed nonsmokers and levels less than or equal to 1.11 ng/mL were defined as unexposed nonsmokers (122). Treatment and outcome information were extracted from subject's medical record. The final population included 1909 self-reported non-smoking women undergoing IVF (122). After adjusting for age, BMI, year of IVF and the type of down-regulation, SHS exposed nonsmokers were significantly associated with decreased live birth rates after IVF, compared to unexposed nonsmokers ((OR: 0.75; 95% CI: 0.57, 0.99) and (aRR: 0.81; 95% CI: 0.66, 0.99)) (122). Previously mentioned limitations remained including generalizability of results given the specific population and the cross-sectional nature which is a weak design to infer causality.

Summary

There is also suggestive but insufficient evidence to support a causal relationship between SHS exposure and SA. However, most studies described earlier did not find a significant association and few that did used designs that are more subject to biases such as confounding and selection bias. The only two prospective cohort studies done were inconclusive and did not use an objective biomarker such as cotinine to verify exposure

status. Of the three studies listed in Table 4 that showed significant associations, two used self-reported exposure information and only one found a significant dose-response effect. Self-reported SHS exposure data are subjected to recall bias and under-reporting, which can create misclassification and null associations. Almost every study measured SHS exposures during pregnancy on SA; however, pre-conception SHS exposure may also affect SA through similar mechanisms. Adjusting for important confounders and taking into account infertility diagnoses among the MAR population is also crucial to understand the biological mechanism of SHS exposure on SA.

Table 4: Studies on Secondhand Smoke Exposures and Spontaneous Abortion

	Study Design	N	ART	Exposure	Outcome	Estimate	95% CI
Ahlborg & Bodin, 1991 (149)	PCS	4,687 women	No	Self-reported	First-trimester fetal loss	2.16[a]	1.23 – 3.81
Windham et al., 1992 (150)	CACO	626 cases 1,300 controls	No	Self-reported	SA	1.50[b]	1.2 – 1.9
Windham et al., 1999 (152)	PCS	~ 5000 women	No	Self-reported	SA	1.01[b]	0.80 – 1.27
George et al., 2006 (151)	CACO	463 cases and 864 controls	No	Plasma cotinine	SA	1.67[b]	1.17 – 2.38
Meeker et al., 2007a (139)	CS of PCS	1449 women	No	Self-reported	SA	0.80[b]	0.30 - 2.14

Meeker et al., 2007b (140)	CS of PCS	921 women	Yes	Urinary cotinine	SA	0.51[b]	0.21–1.24
Benedict et al., 2011 (122)	CS of PCS	1909 women	Yes	Follicular fluid cotinine	Live Birth	0.81[a], 0.75[b]	0.57–0.99, 0.66–0.99
Hyland et al., 2015 (145)	CS	77 805 women	No	Self-reported	SA	1.17[b]	1.05 - 1.30
Salmasi et al., 2010 (153)	MA	9 studies	Both	Self-reported& biomarker	SA	1.17[c]*	0.97 - 1.41
Leonardi-Bee et al., 2011 (154)	MA	6 studies	No	Self-reported and biomarker	SA	1.17[c]*	0.88–1.54
Pineles et al., 2014 (148)	MA	17 studies	Both	Self-reported and biomarker	SA	1.11[c]*	0.95 - 1.31

CS - Cross-sectional, RCS - Retrospective cohort study, PCS - Prospective cohort study, CACO - Case-control, MA - Meta-analysis
a - Adjusted risk ratio
b - Adjusted odds ratio
c - Pooled OR/RR
* - Some individual studies adjusted for confounders

SECTION E - N-Acetyltransferase 2

As previously mentioned, there are over 7,000 chemicals in tobacco smoke and over 70 that are carcinogenic (96). Carcinogens such as polycyclic aromatic hydrocarbons and heterocyclic amines have been found to cause DNA adducts which can lead to mutations, that can initiate carcinogenesis (13,155). N-Acetyltransferase 2

(NAT2) is a vital enzyme used in the metabolism and detoxification of xenobiotic substances, including therapeutic drugs and exogenous chemicals such as the ones found in tobacco smoke (13). NAT2 is primarily expressed in the liver and gastrointestinal tract, and catalyzes the transfer of an acetyl group to compounds, either activating or deactivating it. (13,155).

Single nucleotide polymorphisms (SNPs) within the NAT2 alleles are responsible for determining NAT2 genotype and phenotype status. Seven most frequent SNPs studied are rs1801279 (191G>A), rs1041983 (282C>T), rs1801280 (341T>C), rs1799929 (481C>T), rs1799930 (590G>A), rs1208 (803A>G) and rs1799931 (857G>A) (156). One study compared the accuracy of using different NAT2 SNP genotyping panels and found that a four SNP genotype panel of rs1801279 (191G>A), rs1801280 (341T>C), rs1799930 (590G>A) and rs1799931 (857G>A) produced the highest accuracy in predicting NAT2 acetylator status (156). NAT2 acetylator status is usually coded by the number of variants within the four SNP panel. If the subject had no variants among the four SNPs, they were classified as a rapid acetylator; one variant as an intermediate acetylator and two or more variants as a slow acetylator (156). NAT2 acetylator status explains the effectiveness of therapeutic medicines, differences in cancer risk and how the body metabolizes environmental toxins (14).

In 2011, Sabbagh and colleagues compiled frequency data for the most important NAT2 variants from 128 population samples, to examine whether different dietary patterns and lifestyles can account for inter-population differences in NAT2 variation (14). They grouped together intermediate and rapid acetylators because there had similar acetylation rates and were compared to slow acetylators. The population was categorized

into four major continental areas (Africa, America, Asia and Europe). The highest percentage of genetic variation was found within populations at 87.4% and 8.3% genetic variation across continents (14). The authors found that the slow acetylators accounted for 59% of individuals in Europe and higher percentages of slow acetylators in other places such as the Middle East, Southeast Asia and India (14). On the other hand, the slow acetylator status was uncommon in Northeast Asia, with a prevalence of 18%, which also showed higher percentages of the rapid/intermediate acetylator status (14). Furthermore, the distribution of slow acetylators within Africa and America were highly heterogenous with notable differences between the populations.

Several studies have investigated the association of NAT2 polymorphisms and several cancers such as bladder and colorectal cancer and allergic disorders such as asthma (157) with inconsistent results. A few of those studies further explored effect modification by smoking status. The research showed significant interactions between current smoking and slow acetylation status, with increased risk of bladder cancer (158–161), breast cancer (162,163), colorectal cancer (164,165) and lung cancer (166,167).

NAT2, Smoking, and Fertility-related Outcomes

Some recent studies have examined the relationship between NAT2 polymorphisms and endometriosis: one study found that there was an increased risk of endometriosis among slow acetylators (168), another found no association (169), one meta-analysis showed a significant risk only among Asians (170) and lastly, one study showed that some SNPs may increase risk of endometriosis and others may be protective (171). There have been few studies to date that have examined the interaction of NAT2

polymorphisms with tobacco smoke on fertility. In 2011, Taylor and associates showed that among slow acetylators, current smoking was significantly associated with reduced fecundability (fecundability odds ratio (FOR): 0.34: 95% CI, 0.22, 0.90) among office working women after adjusting for significant confounders (15).

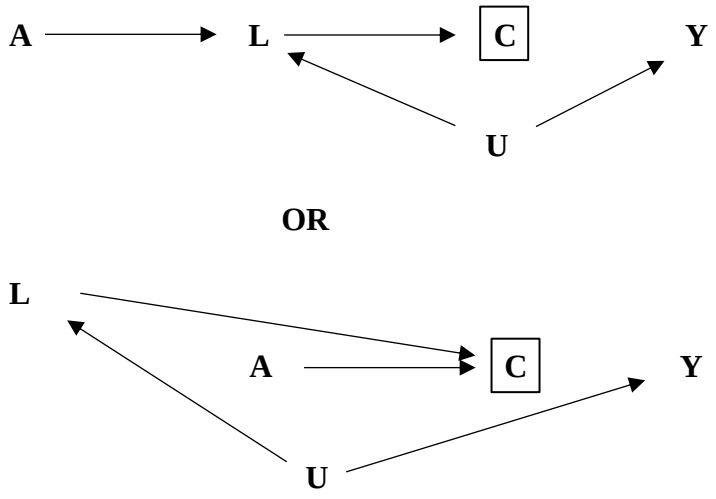
More studies on NAT2 polymorphisms and its interaction with smoking were focused on male infertility. Two recent contradicting case-control studies included Jiang and colleagues who showed no significant association between NAT2 polymorphisms and risk of idiopathic male infertility (IMI) (16), while Trang and associates showed that the rs1799929 and rs1799930 SNPs significantly increased the risk of IMI (17). In 2014, Yarosh and colleagues found that among men with a slow-acetylation status, cigarette smoking was significantly associated with an increased risk of IMI (OR 1.71, 95% CI 1.02, 2.87, P = 0.042) (172). These studies have emphasized the importance of further understanding gene-environmental interactions on fertility outcomes, and the need for more research.

SECTION F - Selection Bias in Prospective Cohort Studies

Selection bias due to differential loss of follow-up, also known as attrition bias, is one major source of potential bias in prospective cohort studies and randomized controlled trials (173). This occurs when loss to follow-up of study participants is jointly associated with both the outcome and the exposure (173). Figure 1 shows two examples of selection bias due to loss to follow-up where the main exposure is smoking (A), the outcome is conception (Y), a set of measured confounders (L) and a set of unmeasured

confounders (U) are displayed in a casual diagram. When subjects are censored (C) either because of loss to follow-up or missing outcome data, they are removed from the study at the time of being censored. This restricts the data to the uncensored observations, which is why C is conditioned on in Figure 1. Conditioning on a collider C as in the bottom diagram in Figure 1 or conditioning on a descendent of a collider L in the top diagram in Figure 1 induces selection bias on the path between exposure A smoking and outcome Y conception (Figure 1) (174).

Figure 1. Example of Selection Bias Due to Censoring



Causal diagrams such as directed acyclic graphs are one way to help to identify potential selection bias. Another method to identify potential selection bias due to censoring is to compare the prevalence of exposures and covariates of non-responders or

partial responders to those who remained in the study and identify significant differences. Sensitivity analyses can also help to assess the potential impact of attrition bias, by assuming a particular distribution of the outcome among all non-responders for example, all censored subjects conceived or all censored subjects did not. However, this technique yields conditional estimates which are difficult to interpret and have shown to be insufficient in correcting selection bias.

A more robust method used to control for selection bias due to censoring is the inverse probability weighting (IPW) technique. In an IPW analysis, a pseudo-population is created by weighting the probability of being followed up in the study given a set of risk factors associated with being lost to follow-up and the outcome. The inverse of the probability of being followed up is computed and included in a weighted analysis for everyone who were lost to follow-up. (173,175). Hence, the subjects who were followed up, account for themselves and those lost to follow-up up with similar characteristics. For example, there are five nonsmokers in a cohort study of the same age, BMI, race and AMH levels, and 4 are lost to follow-up. In this scenario the estimated probability of being followed up (uncensored) is 1/5 = 0.2 and the nonsmoker who was followed up received a weight of 5 (1/0.2). This means that in the pseudo-population, there will be 5 copies of the uncensored nonsmoker to account for the 4 who were censored (176).

In IPW, there are assumptions that must be met in order for valid estimation of casual effect measures. The first assumption of IPW is conditional exchangeability, which assumes that there are no other factors, L, that contribute to the loss of follow-up or selection bias. Sensitivity analyses can be done to test the robustness of estimates by including additional variables, L, that may explain the selection bias (177). The second

assumption is positivity, which assumes a non-zero probability of being censored within every unique exposure combination. And lastly, the model used to estimate the weights must be correctly specified (177). All these assumptions must hold in order to accurately estimate effect that have been adjusted for selection bias due to lost to follow-up.

Rationale

Tobacco smoke exposure is one of the leading preventable causes of mortality and morbidity in the U.S. (11), and its effect on natural and assisted conception and adverse pregnancy outcomes needs to be further examined with more substantial and conclusive evidence. There is a lack of exposure data validation with biomarkers, assessment of the cumulative effects of active smoking and SHS exposure, evaluation of dose-response effects and the use of tobacco smoke exposure at different time points to fully capture the effect and its biological mechanisms. Many studies in the past have not adjusted for all important confounders or explored potential effect modifiers. More large-scale, longitudinal prospective studies are needed with different age groups to fully determine differences throughout the reproductive lifespan. Lastly, reducing the potential for bias, and conducting appropriate statistical tests should be taken into consideration.

The objectives of this study will help to address some of the gaps in the literature and add new, relevant knowledge in regard to the effect of pre-conception tobacco smoke exposures on fertility and pregnancy outcomes and the interaction with NAT2 acetylator status. This study will add new evidence of the association between tobacco smoke exposures and fecundability and SA within in a clinical population. Validation of exposures using cotinine measurement and adjustment of selection bias due to loss of

follow-up can also strengthen the accuracy of estimates. Findings from this study has clinical implications for fertility care and counseling and can help to bring about more public health policies and interventions to reduce the impact of smoking and SHS exposures.

IV. METHODS

Study Design

The data used in this study came from the Louisville Tobacco Smoke Exposure, Genetic Susceptibility and Infertility (LOUSSI) study (Granting institution- Eunice Kennedy Shriver National Institute of Child Health and Human Development; grant number- 1R15HD087911-01; Principal Investigator - Dr. Kira Taylor). Its objective was to estimate the cumulative impact of tobacco smoke exposure and its interaction with NAT2 acetylator status on ovarian reserve and IVF-related outcomes. This study had both cross-sectional and prospective data collection components. The prospective component of the LOUSSI study, examining conception and pregnancy outcomes, was used for this dissertation. Institutional Review Board approval for this study was obtained from the University of Louisville, IRB number: 16.0063.

Study Setting and Subjects

Study participants were recruited from existing and new patients attending the University of Louisville Reproductive Endocrinology and Infertility (REI) clinic. The clinic's clientele included women of reproductive age seeking fertility counselling and/or treatment and who resided in the Louisville and southern Indiana area. The inclusion criteria for the LOUSSI study were all women aged 21 years and older seeking fertility counselling and/or treatment at the University of Louisville REI clinic. Women illiterate

in the English language or found to be pregnant at the time of enrollment were excluded from the study.

Participating physicians and fellows assessed a potential subject's eligibility and invited her to take part in the study. If the patient expressed interest, a research assistant present at the clinic would explain the benefits and risks of the research study and obtain informed consent. Participating subjects were offered compensation with a $25 VISA gift card. In July 2017, after 99 subjects were enrolled, the informed consent was modified to include a section requesting permission to recontact the subject. If the subject agreed to be recontacted, they were asked to leave a phone number, email and mailing address. The final sample size of 264 women were enrolled from November 2016 through May 2018.

Data Collection and Study Instruments

After informed consent was obtained, each subject was assigned a unique identification number which was used to label biological samples, questionnaires and medical report extracts. One patient key recording the subject's name and their respective identification number was made and kept in a secured and locked cabinet at the clinic.

Three sources of data were used to retrieve all information from each subject after informed consent was obtained. A supplemental smoking questionnaire (SSQ; appendix A) was given to each subject to measure and record active (current and lifetime) and SHS exposures (recent and the past year). Information from medical records were extracted to obtain demographic data, medical history and other relevant variables. Laboratory assays were done on urine samples collected to assess cotinine and NAT2 acetylator status. Subjects were then followed to determine whether the subject conceived. Among those

who conceived, pregnancy outcomes were also collected. Patients who gave permission to be recontacted by email, phone or mailing address, were contacted after a period greater than 6 months from enrollment to record any fertility treatments/procedures, and conception and pregnancy outcomes. Medical records were re-reviewed at least 6 months after the end of enrollment to update fertility treatments/procedures and pregnancy outcomes. The subjects who gave permission were personally recontacted twice: September 2018 through January 2019 and July 2019 through September 2019, to obtain further information on conception and pregnancy outcomes.

Study Instrument – Supplemental Smoking Questionnaire

The SSQ (appendix A) was used to estimate current and the previous years' tobacco smoke exposure, for both active and SHS exposures. Questions used were initially adapted from two nationally validated surveys: the National Health and Nutrition Examination Survey III and the National Health Interview Survey but were revised to more precisely address the research questions and the population of the LOUSSI study. There were three questionnaires provided: one each for current smokers, former smokers (women who quit over a month ago), and non-smokers. The current and former smokers' questionnaire contained questions regarding the total amount of years smoking and number of cigarettes or packs smoked per day. The former smoker's questionnaire also included the month and year the participant last smoked.

All questionnaires included three questions to assess SHS exposures (recent and the past year) measured using a four-point scale (never (1), rarely (2), often (3) and every day (4)). Early childhood SHS exposure was assessed by asking subjects "how often were

you exposed to secondhand smoke inside your home while growing up?" There were two questions regarding SHS exposures in the past year, specifically "how often were you exposed to secondhand smoke inside your home in the past year?" and "how often have you been exposed to secondhand smoke inside other places besides your home (example: a friend or relative's house, your workplace, bars or restaurants, in your car or someone else's car) in the past year?" The questionnaires were later modified and approved by the IRB in July 2017 to include questions asking if the participant is living with smoker/s; and if so, how many smokers and their relationship to the smokers. Lastly, the revised version also included a question regarding other sources of nicotine such as nicotine gum and e-cigarettes, since their use would be reflected by cotinine in the urine sample.

Study Instrument – Medical Record Data

The medical record data collection form (appendix B) was created to document important risk factors of both fertility-related outcomes and tobacco smoke exposure, based on the current literature. Research assistants trained by co-investigators and research physicians extracted the information from the subject's intake history form and medical records.

Variables collected from the medical record are listed below:

- Demographics (date of visit, age at enrollment, ancestry or race);
- Anthropometrics and physiological characteristics (height, weight, BMI, and blood pressure);
- Social history and behavioral variables (occupation, marital status, partner status, length of time with current partner, routine exercise; hours of exercise per week,

times per week and type of exercise; routine exposure to chemicals, diet restrictions, how many meals per day, current tobacco use; packs per day with total amount of years smoked; ever smoked 100 cigarettes; alcohol consumption; number of alcoholic drinks per week; caffeine consumption; number of caffeinated drinks per week and use of other drugs);

- Obstetrics and gynecology (OB/Gyn) variables (gravidity, regularity of periods, average menstrual cycle length, age at menarche, consistent ovulation, and history of sexually transmitted infections);
- Reproductive measurement variables (AMH values and dates, AFC values and dates, duration of infertility, type of infertility diagnoses and self-reported PCOS);
- Medically assisted reproduction variables (any MAR procedures done after enrollment, date of procedures, and type of treatment (OI/IUI/IVF/FET));
- Conception (first clinical conception outcome since enrollment, method of the first conception since enrollment, last menstrual period of first conception or date of conception (for OI/IUI/IVF/FET), expected due date);
- Pregnancy outcomes (first pregnancy outcome since enrollment).

Data Source – Cotinine Assays

Each urine sample collected at enrollment, labelled with the corresponding patient's identification number was divided into two samples: one for cotinine analysis and the other for DNA extraction to perform NAT2 genotyping. All urine samples for cotinine analysis were stored at 4 degrees Celsius for up to 72 hours and then aliquoted

and frozen at -80 until the day of the cotinine assay. Cotinine assays were done at the University of Louisville's Medical Dental Research Building.

Cotinine ELISA kits (Calbiotech, Spring Valley, CA) were used to measure the patient's cotinine levels to differentiate active, passive and no exposure to tobacco smoke in the past 24 to 72 hours. Standard controls and a negative control (water) were done in triplicate, while patients' samples were either assayed in triplicate or duplicate. A spectrophotometer was used to measure the absorbances of the standards, controls and samples. The mean, standard deviation and relative standard deviation were calculated for all absorbances. Outliers among triplicate samples were removed, and if the relative standard deviation was greater than 0.1, the sample was re-assayed in triplicate.

Firstly, a standard curve was constructed by plotting the mean absorbances of the cotinine standards versus the cotinine standard concentrations on a line graph. Using the fitted standard curve, cotinine concentrations of each subject was derived from their corresponding average absorbance. Urinary cotinine concentrations were estimated to the nearest 0.50ng/mL and the maximum detectable level of cotinine for this assay kit was 100ng/mL. All assay analyses were done by two research assistants and compared to increase the accuracy and reliability of results.

Data Source – Genotyping Assays

DNA was extracted using the ZR Urine Isolation Kit ™ (Irvine, CA, USA). DNA extractions were done on a weekly basis then stored at -20 degrees Celsius until time of genotyping. NAT2 genotyping was done at the University of Louisville's Clinical and Translational Research building.

NAT2 acetylator status was assessed using a four SNP genotype panel with the assistance of a trained laboratory technician. Twenty percent or more of the samples in each batch were done in duplicate along with a negative control (blank or water). If the genotype status was not clear from the four SNPs due to a lack of DNA amplification or contamination, samples were repeated in duplicate if there were remaining DNA. All assay analyses were done by two research assistants and compared to increase the accuracy and reliability of results.

Different alleles of NAT2 have been associated with rapid and slow acetylation. The four SNPs (rs1801279(191G>A), rs1801280(341T>C), rs1799930(591G>A), rs1799931(857G>A)) were coded by the number of variants. If the subject had no variants among the four SNPs, they were classified as a rapid acetylator; one variant classified them as an intermediate acetylator and two or more variants as a slow acetylator (Table 5). Intermediate and rapid acetylators were grouped together as they have been shown in the literature to have similar acetylation characteristics (14).

Table 5. Example of NAT2 Phenotype Status Using Four (4) SNPs

SNP	191	341	590	857	# of variant alleles	Phenotype
Patient A	0	0	0	0	0	Rapid
Patient B	0	0	1	0	1	Intermediate
Patient C	1	0	1	0	2	Slow
Patient D	1	1	0	1	3	Slow

Study Instrument – Recontact Questionnaire

Subjects who agreed to be recontacted by either phone, email or mailing address as indicated on their informed consent were contacted at least 6 months after enrollment to obtain information on fertility treatments/procedures, and conception and pregnancy outcomes. If an email address was provided, an initial email was sent out to notify subjects of the recontact and they were given the option to answer the questions by email or reply with convenient times to call via phone. The next method was phone call, if a phone number was given and working. Three attempts were made, leaving a brief voicemail if no one answered. If no contact was made, another email with the questions was sent. And lastly, if mailing addresses were given and up to date, a letter was mailed using the U.S. postal service as a final attempt to recontact the patient.

Using the recontact questionnaire (appendix C), they were first asked "Have you conceived since enrollment in the LOUSSI Study(yes/no)". If the subject answered yes, they were then asked whether it was a natural conception or if they used MAR procedures. The type of MAR procedure used and its date, if conception was attained, the last menstrual cycle date or conception date, the due date of the first conception, whether or not they were currently pregnant, and the pregnancy outcome were recorded. If the subject answered that they had not conceived since enrollment, they were asked whether or not they were still trying to conceive. If they were no longer trying, they were then asked how long after enrollment did they stop trying and if they were using any form of contraception. Subjects were recontacted twice after enrollment ended, firstly from September 2018 through January 2019 and then from July 2019 through September 2019

to obtain further information on conception and pregnancy outcomes if they gave permission.

Data collection and entry, as well as assay analyses for all sources of data were done by two trained research assistants and compared for accuracy. Discrepancies were resolved by reviewing the medical records and hard copies of the SSQ.

Outcome Assessment

Conception outcomes were either extracted from medical records or from the patients who were successfully recontacted. For this study, the first conception and corresponding pregnancy outcome since enrollment were used in the analysis.

Follow-up time measured in months or TTC, for those who conceived was prospectively observed and measured by calculating the number of months between enrollment and first conception, including the month of enrollment and the month of conception. This was a straightforward calculation for spontaneous conceptions, by subtracting the month of enrollment from last menstrual cycle and adding 2. For subjects who had MAR procedure(s), their TTC was calculated in cycles/intervals at risk with MAR cycles used as a time-varying covariate from enrollment to first conception.

Among those who did not conceive and did not use MAR during the study period, follow-up time measured in months was calculated from enrollment to either the date of the last visit at the clinic in the medical record that indicated that the subject was not pregnant or month of last personal re-contact, if the permission was given. For subjects who had MAR procedure(s) and did not conceive, their follow-up time was calculated in intervals at risk with MAR cycles as a time-varying covariate from enrollment to the

same endpoints stated above based on whether or not they gave permission to re-contacted.

If subjects were seen only once at the clinic, were not personally recontacted, and did not have at-risk follow-up time, they were removed from the TTC analysis. Months with contraception use and months noted on subject's medical record that intercourse was advised against, were excluded from the follow-up time because the subjects were theoretically not at risk for pregnancy during those months. Same-sex couples who did not use MAR after enrollment were also excluded from the TTC analysis.

Pregnancy outcomes of the first conception since enrollment were either extracted from medical records or retrieved from successful recontact. The answers included live birth, SA, ectopic pregnancy, molar pregnancy and stillbirth or fetal death. Live birth and SA were used in the analysis due to lack of events in the other categories.

Exposure Assessment - Current, Active Smoking

Current, active smoking was measured and assessed using the SSQ and cotinine levels. A current, active smoker by the SSQ was defined when the subject self-reported as a current smoker or if the subject reported quitting smoking under a month ago. Also, if any subject had a urinary cotinine level of 100ng/mL, they were defined as a current, active smoker, regardless of which questionnaire was filled out, otherwise were defined as a nonsmoker.

A cumulative lifetime smoking variable using pack-years was created by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. For example, 1 pack-year is equal to smoking 1 pack per day for 1

year. If the participant reported number of cigarettes per day, that number was initially divided by 20 to convert it to packs. The pack-years variable was further categorized into three groups. Subjects with no pack-years (referent) was compared to pack-years of 5 or less and pack-years greater than 5. Self-reported smokers and former smokers provided either the number of cigarettes smoked per day or the number of packs smoked per day, if it were the latter, that number was multiplied by 20. The total number of cigarettes per day for all subjects was also calculated and grouped into three categories: 0 cigarettes (referent), 9 cigarettes or less and greater than or equal to 10 cigarettes.

Exposure Assessment – SHS Exposure (Recent and Past Year)

Recent and the past year's SHS exposure was assessed for only women defined as nonsmokers, using the objective measure described above. Current, active smokers were excluded from the SHS analysis. To examine current SHS exposure, cotinine values were categorized into three groups. Subjects with cotinine values of 0 ng/mL were place in an unexposed group (referent), values greater than 0 ng/mL and less than 4.0 ng/mL were considered as low exposure; and cotinine values greater than or equal 4.0 ng/mL and less than 100 ng/mL were considered highly exposed to SHS.

Using the SSQ, a score was created for adult SHS exposure in the past year. The two SHS exposure questions on the SSQ "how often were you exposed to secondhand tobacco smoke in your home in the past year?" and "how often were you exposed to secondhand tobacco smoke in other places such as work, restaurants, friends and family's homes in the past year?" were combined to create the score. The responses to each question were given a numerical value as follows: never (1), rarely (2), often (3) and

every day (4) and summed. The combined score for adult SHS exposure in the past year variable ranged from 2 to 8 and was grouped into 4 categories. A description of how the SHS exposure in the past year variable was categorized is presented in Table 6.

Table 6. Description of SHS Exposure in the Past Year Variable from SSQ

Score	Answers to 2 SHS Questions from SSQ (SHS in the home, SHS in other places)	4-Level Group	Final Grouping
2	(never, never)	None (0)	Low SHS exposure
3	(never, rarely)	Low (1)	
4	(never, often) or (rarely, rarely)	Intermediate (2)	
5	(never, everyday) or (rarely, often)	Intermediate (2)	
6	(rarely, everyday) or (often, often)	High (3)	High SHS exposure
7	(rarely, often)	High (3)	
8	(everyday, everyday)	High (3)	

Exposure Assessment – Combined Effect Exposure

A combined effect variable using the objective, current active smoking variable and the final SHS exposure variable from the SSQ was created. The new combined effect variable included three groups: non-smokers with low SHS exposure (referent), non-smokers with high SHS exposure and current, active smokers. Table 7 shows the treatment of all exposure variables used in this study.

Table 7: Exposure Variable Definitions and Treatment in Modeling

Variable	Definition
Current, active smoking Dichotomous	Smoker (1) = Cotinine level of 100 ng/mL or Self-reported as a Current Smoker Non-Smoker (0) = Cotinine level < 100 ng/mL and No Self-report of Current Smoker (Referent)
Pack-years Categorical	0 = 0 pack-years (Referent) 1 = ≤ 5 pack-years 2 = > 5 pack-years
Total Number of cigarettes Categorical	0 = 0 cigarettes per day 1 = ≤ 9 cigarettes per day 2 = ≥10 cigarettes per day
Cotinine* Categorical	0 = 0 ng/mL (Referent) 1 = 0 < cotinine ≤ 4 ng/mL 2 = ≥ 5 ng/mL
Adult SHS exposure in the past year* Categorical No/Low Intermediate/High	Adult SHS Score ≤ 3 (Referent) Adult SHS Score ≥ 4
Combined effect exposure (Current, Active Smoking and Adult SHS Score) Categorical	Non-smoker with Low Adult SHS exposure (Referent) Non-smoker with High Adult SHS exposure Current, Active Smokers

* - Among objectively defined non-smokers

Important Covariates

Relevant covariates were chosen based on a theorized directed acyclic graph (DAG) developed from *a priori* knowledge and were either collected at time of enrollment if it was the patient's first visit or extracted from the medical record at time of the first clinic visit. These included age at the time of enrollment, BMI recorded by the

clinic at time of enrollment, ethnicity/race, gravidity, age of menarche, average cycle length, menstrual cycle regularity, history of previously diagnosed sexually transmitted diseases, marital status, current routine exercise, alcohol consumption, caffeine consumption, AMH level, self-reported length of infertility, ART treatment cycles, type of ART treatment, and infertility diagnoses.

Age at enrollment, BMI at enrollment, gravidity, average cycle length, age of menarche, self-reported length of infertility and AMH levels were recorded continuously. Menstrual cycle regularity, history of past diagnosed sexually transmitted diseases, marital status, routine exercise, alcohol consumption, caffeine consumption and MAR cycle were dichotomized no or yes. Ethnicity was recorded as Caucasian, African American, Asian/Pacific Islander, American Indian/Alaska Native, Middle Eastern, Hispanic/Latin American and other. Lastly, the type of infertility diagnosis was collected and included unexplained, tubal, uterine, ovarian, male factor, PCOS, other, unknown and none. The infertility diagnoses were not mutually exclusive, and many women had more than one diagnosis.

Data Analysis

Descriptive Statistics

All statistical analyses were performed using R Studio version 1.2.5 (Boston, MA, USA). Descriptive statistics were calculated for covariates to examine their distributions. Univariate analyses were conducted to examine the crude association between covariates and exposure variables from Table 7 within three different populations (all individuals, MAR users, and non-MAR users). This was done using Chi-square/Fisher exact test for

categorical variables and Student's T-test for normally distributed, continuous variables or Wilcoxon rank-sum for non-normally distributed, continuous variables. Significant differences were defined with p-value <0.05.

Covariates in the final multivariable logistic regression and survival analysis models were initially chosen using both theory and data-based methods. Covariates associated with the exposure with a p-value of less than 0.2 from univariate analyses were included. However, age and BMI were retained in all models regardless of p-value given their well-established associations with fertility or birth outcomes. Table 8 shows the description and treatment of all chosen covariates used in the final models. Age was dichotomized at the median, which demonstrated a shift in fecundability in this population; BMI was dichotomized at the WHO's obesity threshold (30 kg/m_2). Age and BMI had been previously shown to have a non-linear association with fecundability in past studies. Race was dichotomized into African American and other races because it showed the most significant association with pregnancy within the dataset compared to other categorizations.

Table 8: Covariate Definitions and Treatment in Modeling

Variable	Type of variable	Definition
Age (years)	Dichotomous (at median)	0 = ≤ 33 1 = > 33
BMI (kg/m_2)	Dichotomous Continuous (Aim 2)	0 = ≤ 30 1 = > 30
MAR cycle	Dichotomous	0 = No 1 = Yes
Gravidity	Dichotomous (Aim 2)	0 = no prior pregnancies 1 = ≥ 1 prior pregnancy
	Categorical (Aim 1)	0 = no prior pregnancies

		1 = 1 prior pregnancy 2 = ≥ 2 prior pregnancies
Marital Status	Dichotomous	0 = No 1 = Yes
Race	Dichotomous	0 = Other races 1 = African American
Alcohol Use	Dichotomous (Aim 2)	0 = No 1 = Yes
NAT2 Acetylator Status	Dichotomous	0 = Fast/Intermediate 1 = Slow
MAR Type*	Dichotomous	0 = OI/IUI 1 = IVF/FET

* - Used only when modeling MAR cycles only population

Multivariable Models

Survival Analyses - Cox Proportional Hazard Regression Models

The dataset for survival analyses was constructed in a counting process structure to include MAR cycle as a time-varying covariate. Each row in the dataset indicated a time interval in months at risk for conception, categorized by whether or not the month was a MAR cycle. The coxph and surv function in the survival package was used to run Cox models. A cluster term for participant's ID was included in the model to show that participants may have multiple rows of data and produced estimates with robust standard errors to address non-independence. Start time was defined as the month of enrollment (time 0) and the stop time referred to either the month the subject had the event (conception) or the month the subject was censored. Month of censorship was defined by either the date of last re-contact, the date of last medical record visit, or the date the subject reported last actively trying to conceive.

A statistical test using the cox.zph function was used to assess the proportional hazards assumption on all variables in the full models. The test examined the correlation between survival time (or ranked survival times) and Schoenfeld residuals. A p-value less than 0.05 indicated that the proportional hazards assumption was violated and the Schoenfeld residuals were dependent on survival time. If this were the case, the appropriate models were adjusted for an interaction term of the variable in violation and time.

All models were evaluated for multicollinearity using variance inflation factors using the vif function in the rms package, before and after confounding assessment. Confounders were assessed using a combination of theory and data-based approaches. The full models were initially fitted and variables that changed the estimate of the main exposure by 10% when removed from the model were defined as data-based confounders, and hence remained in the model. Age and BMI, however, were retained in all models because of the consistent and strong evidence of associations with fertility in the literature. Statistically significant variables were defined by a p-value less than 0.05.

There were six different exposure variables, and each were examined within the following sub-populations: all follow-up time, MAR cycles only, and natural conception (NC) follow-up time only. Women in the natural conception follow-up sub-population contributed intervals of at-risk follow-up time for natural conception and did not use MAR procedures during these intervals. The number of NC follow-up was estimated using calendar months, as there was not data on ovulation available.

Among all follow-up time models, the time-varying MAR cycle variable (yes/no) was included as a covariate. In the model that included MAR cycles only, the MAR type

in the MAR cycle (IVF/FET vs. OI/IUI) was included as a time-varying covariate, as it changed over intervals. Within these two sub-populations, models were analyzed using Extended Cox regression because they included a time-varying covariate; whereas, the NC follow-up time models used Cox proportional hazards regression. Hazard ratios of fecundability also called fecundability ratios (FR) and 95 % confidence intervals were estimated and reported. Main exposure variables with two or more categories such as cotinine levels among non-smokers and the combined effect variable were further examined ordinally for a dose-response effect, and their respective p-values for trend were reported.

Specific Aim 1a: Current, Active Smoking and Fecundability

The effect of current, active smoking on fecundability was assessed using survival analysis. The objectively measured smoking variable was used to compare current, active smokers to non-smokers. The effect of cumulative lifetime smoking on TTC was also analyzed, using pack-years as exposure. Fecundability ratios and 95% CI's were calculated and adjusted for potential confounders.

Specific Aim 1b: Recent and Past Year's SHS Exposure and Fecundability

The effect of recent and past year's SHS exposure on fecundability was assessed using survival analysis among non-smokers. Firstly, current SHS exposure was assessed using urinary cotinine levels collected at enrollment. Secondly, the SHS exposure scores from the SSQ was used to compare low and high SHS exposure in the past year. Fecundability ratios and 95% CI's were calculated, adjusted for potential confounders.

Specific Aim 1c: Combined Effect of Active Smoking With SHS Exposure and Fecundability

The combined effect variable using current, active smoking and SHS score in the past year on fecundability was assessed using survival analysis. Current smokers and nonsmokers with high SHS exposure was compared to nonsmokers with low SHS exposure. A dose-response effect or trend test was estimated by modelling the 3-level categorical variable, ordinally. Fecundability ratios and 95% CI's for were calculated, adjusted for potential confounders.

Specific Aim 1d: Interaction of Active Smoking With NAT2 Acetylator Status and Fecundability

Effect modification by NAT2 acetylator status on current smoking and fecundability was examined by adding an interaction term for current, active smoking and NAT2 acetylator status to the full model from the survival analysis in specific aim 1a. Fecundability ratios and 95% CI's were calculated, adjusted for potential confounders. Significant interaction was defined with a p-value <0.05 for the interaction term.

Multivariable Logistic Regression Models

The effect of tobacco smoke exposures on spontaneous abortion versus live birth was examined using multivariable logistic regression, among women who conceived. Pregnancy outcomes variable was defined as either a live birth (0) or spontaneous

abortion (SA)/miscarriage (1). The different tobacco smoke exposures from Table 7, were analyzed with the pregnancy outcomes among all women who conceived.

Variables chosen for the full models included age, BMI, gravidity, marital status, race and alcohol consumption, because of its known association with spontaneous abortion. All models were evaluated for multicollinearity using variance inflation factors with the vif function in the rms package, before and after confounding assessment. Similar to Aim 1, age and BMI were retained in all models. Dose-response relationships were assessed for exposure variables with more than 2 categories, by treating the variable ordinally and their respective p-values for trend were reported.

Specific Aim 2a: Current, Active Smoking and Spontaneous Abortion

The effect of current, active smoking on the probability of SA was assessed using multivariable logistic regression, comparing current active smokers to non-smokers. A dose-response relationship was also assessed, using the total number of cigarettes per day. The cumulative lifetime smoking on probability of pregnancy outcome was analyzed using pack-years. Odds ratios and 95% CI's were calculated, adjusted for potential confounders.

Specific Aim 2b: Recent and Past Year's SHS Exposure and Spontaneous Abortion

The effect of recent and past year's SHS exposure among nonsmokers on probability of SA was assessed using multivariable logistic regression. Firstly, current SHS exposure was assessed using urinary cotinine collected at enrollment, grouped into 3 categories. Secondly, SHS exposure in the past year variable from the SSQ was assessed

comparing high SHS exposure to low SHS exposure in the past year. Odds ratios and 95% CI's were calculated, adjusted for potential confounders.

Specific Aim 2c: Interaction of Active Smoking With NAT2 Acetylator Status and Spontaneous Abortion

Effect modification by NAT2 acetylator status on probability of SA was examined by adding an interaction term for current, active smoking and NAT2 acetylator status to the full multivariable logistic regression model from Aim 2a. Odds ratios and 95% CI's were calculated, adjusted for potential confounders.

Specific Aim 2d: Combined Effect of Active Smoking and SHS Exposure and Spontaneous Abortion

The combined effect variable using current, active smoking and SHS score in the past year on probability of SA was assessed using multivariable logistic regression. Current smokers and nonsmokers with high SHS exposure was compared to nonsmokers with low SHS exposure. A dose-response effect or trend test was estimated by modelling the 3-level categorical variable, ordinally. Odds ratios and 95% CI's were calculated, adjusted for potential confounders.

Selection Bias

Specific Aim 3a: Evaluation of Selection Bias due to Loss to Follow-up

The full dataset included women that contributed no follow-up time (n=257). The dataset was divided into two groups: Group 1 contained women that were not asked

permission to be personally followed up and Group 2 contained the remaining women who were asked permission for personal follow-up. The groups were then each stratified by the type of follow-up they received: either no follow-up, follow-up through medical record only or personal follow-up by phone, email or mailing address only.

Characteristics of women in each group were then compared by the type of follow up they received to evaluate the potential for selection bias due to loss to follow-up also known as attrition bias or differential loss to follow-up. Group 1 compared women with no follow-up data (n=21) to those with only medical record follow-up (n=74), whereas group two compared no follow-up (n=7) to medical record follow-up only (n=35) and to personal follow-up only (n=120). Differences between types of follow-up within each group were assessed using Chi-square/Fisher exact test for categorical variables and Student's T-test for normally distributed, continuous variables or Wilcoxon rank-sum for non-normally distributed, continuous variables. Significant differences were defined with p-value <0.05. If there were no significant differences among Group 2 between medical record follow-up only and to personal follow-up only, then Group 1 and Group 2 would be combined and women with no follow-up would be compared to women with follow-up.

Specific Aim 3b: Inverse Probability Weighting

Current smoking was significantly associated with attrition, in that smokers were more likely to be censored (lost to follow-up). Inverse probability weighting (IPW) was used to account for differential loss to follow-up and for the aforementioned confounding variables to examine the associations between current smoking status and the

development of two outcomes: conception as a dichotomous variable (yes/no) and fecundability as the probability of conception over the entire follow-up period.

IPW: Conception Outcome - Dichotomously

To adjust for confounding using IPW, a pseudo-population was made by weighting each subject by the reciprocal (inverse) of the conditional probability of being a smoker conditional on MAR cycle, age, BMI, gravidity, marital status and race, such that in the pseudo-population there is no relationship between smoking and the aforementioned confounders. The denominator of the stabilized inverse probability weights was estimated fitting a logistic regression of being a smoker, conditioned on MAR cycle, age, BMI, gravidity, marital status and race. While the numerator of the stabilized inverse probability weights was estimated by fitting a logistic regression model for the probability of being a smoker with only the intercept.

The geeglm function in the geepack package in R was used to fit a generalized estimating equations (GEE) model using a robust variance estimator to allow for correlated observations created by weighting. The GEE model was fitted to examine the association of smoking on conception in the weighted pseudo-population adjusted for confounding. The inverse-probability weighted ORs adjusted for confounding, along with 95% CIs were reported and compared to the estimates from the original aims.

Selection bias due to loss to follow-up was also adjusted for using IPW. A pseudo-population was made by weighting each subject by the reciprocal (inverse) of the probability of being uncensored conditional on age, BMI, gravidity, race and current smoking. The denominator of the stabilized inverse probability weights was estimated by

fitting a logistic regression model for the probability of being uncensored, conditional on current smoking, age, BMI, gravidity and race. While the numerator of the inverse probability weights was estimating using a logistic regression model for the probability of being uncensored, conditioned on only smoking.

The stabilized inverse probability weights estimated from confounding and selection bias due to loss of follow-up were multiplied, to adjust for them simultaneously. The GEE model was fitted to examine the association of smoking on conception in the weighted pseudo-population adjusted for both confounding and selection bias. The weighted ORs adjusted for both confounding and selection bias to loss of follow-up, along with 95% CIs were reported and compared to the estimates from previous aims. These steps were then repeated to adjust for confounding and selection bias due to loss to follow-up among non-MAR users and estimates were documented and compared.

IPW: Fecundability

Confounding and selection due to loss of follow-up within the fecundability data were adjusted for separately and jointly using IPW, with the ipwtm function in the ipw package in R. The function was used to estimate stabilized inversed probability weights at each time interval at-risk during follow-up, taking into account fixed and time-varying confounders. The longitudinal data was coded in the counting process format and was used to fit Cox proportional hazard models.

Stabilized inverse probability weights to adjust for confounding only were estimated by fitting two Cox proportional hazard models. The denominator of the stabilized inverse probability weights was estimated from a model that examined

probability of smoking at each time-interval, given MAR cycle, age, BMI, gravidity, marital status and race. While, the numerator of the stabilized inverse probability weights was estimated by fitting a logistic regression model for the probability of being a smoker with only the intercept at each time-interval. The stabilized time-interval-specific weights were then used to fit a marginal structured model (MSM) that assessed the effect of smoking on fecundability, to estimate measures of association adjusted for confounding only. Inverse-probability weighted FRs adjusted for confounding, along with 95% CIs were reported and compared to the estimates from original aims.

Selection bias due to loss of follow-up was adjusted for using stabilized inverse probability of censoring weights similarly to the weights used for confounding adjustment. The denominator of the stabilized inverse probability weights was estimated by fitting the regression model for the probability of being uncensored at each time-interval, conditional on current smoking, age, BMI, gravidity and race. While the numerator of the inverse probability weights was estimating using a regression model for the probability of being uncensored, conditioned on only smoking at each time-interval. Stabilized inverse probability of censoring weights were then used to fit a MSM that assessed the effect of smoking on fecundability.

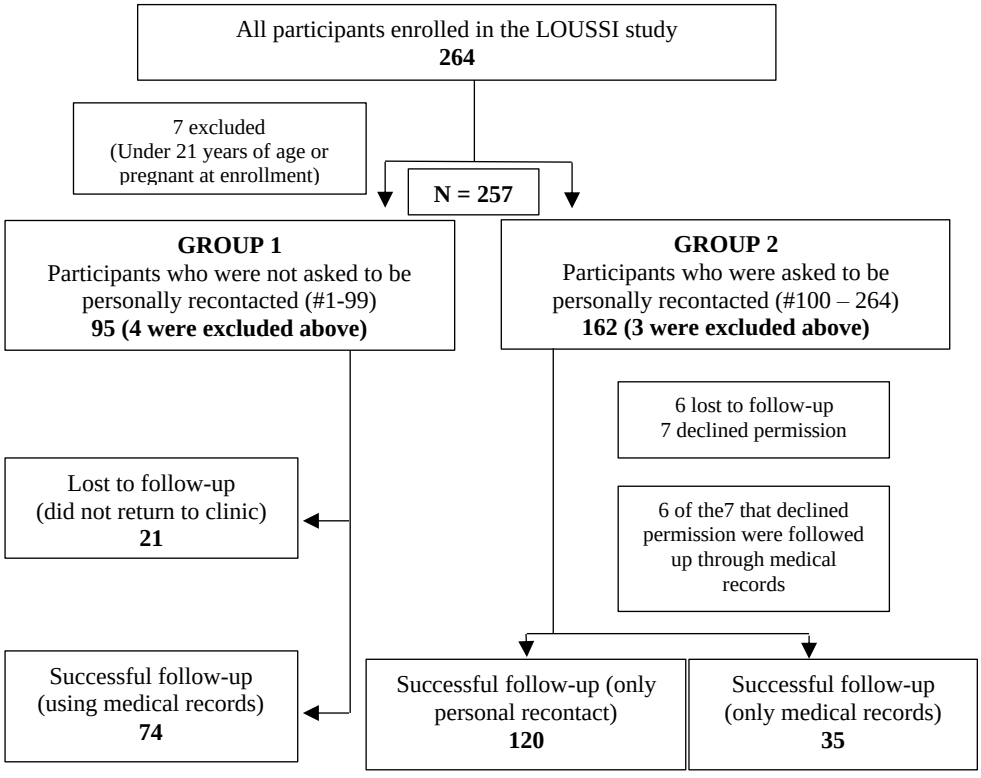
The stabilized inverse probability weights from confounding and selection bias due to loss of follow-up were multiplied to adjust for them simultaneously. The stabilized time-interval-specific weights were then used to fit a MSM that assessed the effect of smoking on conception, to estimate measures of association adjusted for confounding and selection bias due to loss of follow-up. Inverse-probability weighted FRs adjusted for both confounding and selection bias to loss of follow-up along with 95% CIs were

reported and compared to the estimates from previous aims. These steps were then repeated to adjust for confounding and selection bias due to loss to follow-up among NC follow-up time and estimates were documented and compared.

V. RESULTS

Among 264 participants enrolled in the LOUSSI study, seven women were excluded: three were under the age of 21 and four were found to be pregnant at the time of enrollment. Out of the remaining 257 women, 229 (89.1%) were successfully followed up after baseline either through information extracted from medical records and/or if they gave permission through personal re-contact via phone, email or postal mail. Figure 2 illustrates the follow-up methods of participants with and without permission to recontact.

Figure 2: Study Flow Diagram Showing Follow-Up Data among Participants



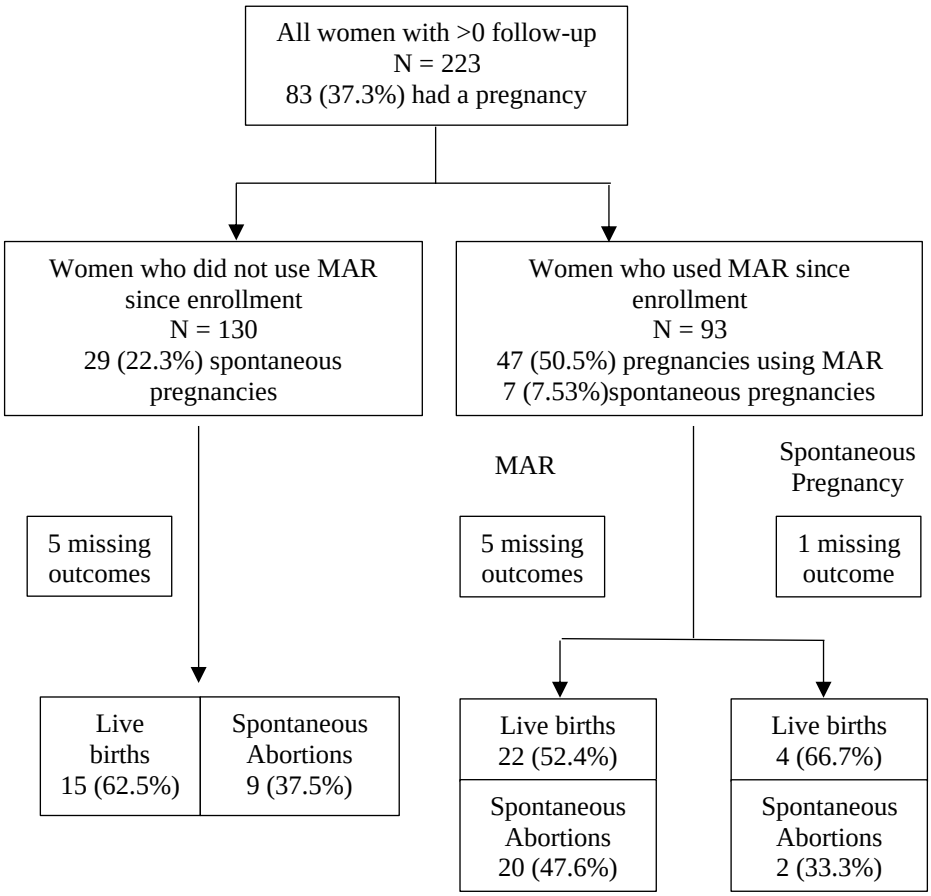
Of the 120 women who were personally recontacted from Group 2, five women reported that they did not continue to pursue conception since enrollment and one women did not provide TTC data at recontact, hence these 6 women did not contribute at-risk months and were removed. These 114 women in addition to the women followed up by medical record only (74 women from the Group 1 and 35 women Group 2), brought the final sample size for the fecundability analyses to 223 women. These 223 women were followed up to 28 months and contributed a total of 1,967 at-risk months (median: 7 months; Q_1-Q_3: 3 - 15 months).

Figure 3 shows the distribution of conception and pregnancy outcomes by MAR. Among the 223 women with TTC data, 83 (37.2%) had a documented and/or self-reported pregnancy since enrollment. Among the total 83 pregnancies reported, there were documented or self-reported pregnancy outcomes for 72 pregnancies. Of the eleven women with missing pregnancy outcomes, 5 were still pregnant at the end of follow-up from Group 2 and 6 were from Group 1 that did not return to the clinic. These eleven women were excluded from the SA analysis. Forty-one (56.9%) of the documented pregnancy outcomes were live births, and 31 (43.1%) were SAs. Among the 130 (58.3%) women who did not use any MAR treatment since enrollment, 29 (22.3%) had spontaneous pregnancies. Of the 93 women who had at least one MAR treatment since enrollment, 47 (50.5%) had successful MAR treatments resulting in a pregnancy and 7 (7.53%) had a spontaneous pregnancy after unsuccessful MAR treatments.

Within the study population (n=223), fifty-four (24.2 %) were verified as current, active smokers, 112 (50.2%) were nonsmokers with low SHS exposure, and 57 (25.6%)

were nonsmokers with high SHS exposure. Cotinine levels were correlated with self-reported SHS exposure ($r = 0.39$, $p < 0.001$) among all women.

Figure 3: Flow Diagram of Conception and Pregnancy Outcomes



Descriptives Statistics

Descriptive analyses were conducted for four different tobacco smoke comparisons of active and second-hand smoke exposures within the full study population (n=223). The four different smoking status comparisons included: 1) current smokers (n= 54) versus former or never-smokers (n=169); 2) current SHS exposure via urinary cotinine among nonsmokers with any exposure (cotinine >0 ng/mL) (n=78) vs.

unexposed (cotinine=0 ng/mL, n=82);3) self-reported SHS exposure in the past year via the SSQ score among nonsmokers: high SHS exposure = score ≥ 4 (n=57) compared to low SHS exposure = score ≤ 3 (referent, n=112); and 4) nonsmokers with low SHS exposure score (referent, n=112) were compared to nonsmokers with high SHS exposure score (n=57) and current smokers (n=54). We additionally stratified analyses by MAR to examine women that used MAR after enrollment (n=130) and women who did not use MAR prior enrollment (n=93) (Appendix D Table S1-8).

In all descriptive tables, categorical variables are presented with N(%), normally distributed continuous variables are presented with their mean ± standard deviations (SD) and continuous variables that did not follow a normal distribution are presented with their median and interquartile range(Q_1-Q_3).

1) Current Active Smokers Compared to Current Nonsmokers

Table 9 shows the characteristics of all 223 women with follow-up time, stratified by current active smoking. Smokers were defined as women with a cotinine level of >=100 ng/mL or who had self-reported as a current smoker. A nonsmoker was defined as anyone with a cotinine level < 100 ng/mL and did not self-report as a current smoker.

Current smokers had longer average menstrual cycle lengths (P = 0.01), were more likely to have a previous STD (P = 0.07), less likely to be married (P <0.001), less likely to exercise (P = 0.10), more likely to be of African American race (P <0.001), more likely to not use MAR (P <0.001) and more likely to have a SA (P = 0.03) compared to nonsmokers. Cotinine levels were also significantly correlated with current active smoking (r=0.98, p-value <0.001). Descriptive statistics comparing current

smokers and nonsmokers stratified by MAR can be found in the Appendix D (Table S1-2).

Table 9 : Characteristics among Women with Follow-up Data Stratified by Current Smoking (N=223)

	Nonsmoker N = 169	Current Smoker N = 54	P - value
Cotinine Levels, mg/dL median (Q_1-Q_3)	0.00(0.00-4.00)	100(100-100)	<0.001
Cotinine Categories, n (%)			<0.001
0 mg/dL	82(51.25)	0(0.00)	
<100 mg/dL	78(48.75)	2(3.85)	
100mg/dL	0(0.00)	50(96.15)	
Age in years, median (Q_1-Q_3)	33.00(28.00-37.00)	32.50(27.25-36.00)	0.48
Age in years, n (%)			0.59
≤33	91(53.85)	32(59.26)	
>33	78(46.15)	22(40.74)	
BMI, median (Q_1-Q_3), (kg/m^2)	28.54(24.82-35.17)	30.36(26.77-34.46)	0.22
BMI Category kg/m^2, n (%)			0.31
<25 (Underweight and Normal)	46(27.22)	11(20.37)	
25 - 29.9 (Overweight)	49(28.99)	13(24.07)	
≥ 30 (Obese)	74(43.79)	30(55.56)	
BMI kg/m^2, n (%)			0.18
≤30	95(56.21)	24(44.44)	
>30	74(43.79)	30(55.56)	
Gravidity, n (%)			0.44
0	74(43.79)	19(35.19)	
1	38(22.49)	12(22.22)	
>=2	57(33.73)	23(42.59)	
Regular Period, n (%)			1.00

No	58(34.32)	18(33.33)	
Yes	111(65.68)	36(66.67)	
Average Cycle Length (days), median (Q_1-Q_3)	28.00(27.50-30.00)	29.50(28.00-30.00)	0.01
Age of Menarche, in years, median (Q_1-Q_3)	13.00(12.00-14.00)	12.00(11.00-13.00)	0.11
Previous STD, n (%)			0.07
No	107(63.69)	25(48.08)	
Yes	61(36.31)	27(51.92)	
Marital Status, n (%)			<0.001
No	46(27.22)	37(68.52)	
Yes	123(72.78)	17(31.48)	
Routine Exercise, n (%)			0.10
No	67(41.36)	28(56.00)	
Yes	95(58.64)	22(44.00)	
Alcohol Use, n (%)			0.75
No	97(57.40)	29(53.70)	
Yes	72(42.60)	25(46.30)	
Caffeine Use, n (%)			0.52
No	34(20.36)	8(15.09)	
Yes	133(79.64)	45(84.91)	
Race, n (%)			<0.001
Other	128(75.74)	27(50.00)	
Black	41(24.26)	27(50.00)	
AMH (ng/mL), median (Q_1-Q_3)	2.49(1.11-5.01)	2.48(1.52-5.66)	0.55
Length of Infertility (years), median (Q_1-Q_3)	2.00(1.00-4.46)	2.50(1.33-5.50)	0.16
Treatment Cycles, n (%)			<0.001
0	87(51.48)	43(79.63)	
1	35(20.71)	3(5.56)	
2 and more	47(27.81)	8(14.81)	
NAT2 Acetylator Status, n (%)			0.32
Rapid	84(57.93)	33(67.35)	
Slow	61(42.07)	16(32.65)	

Pregnant, n (%)				0.14
	No	101(59.76)	39(72.22)	
	Yes	68(40.24)	15(27.78)	
Pregnancy Outcome, n (%)				0.03
	Live Birth	37(63.79)	4(28.57)	
	SA	21(36.21)	10(71.43)	
Total Follow-up Time (years), median (Q_1-Q_3)		6.00(3.00-14.00)	7.00(2.25-15.00)	0.94

2) Secondhand Smoke Exposure – Recent SHS Exposure (Urinary Cotinine) among Nonsmokers

Of the 169 nonsmoking women, 9 did not provide urine samples which left a final sample size of 160 nonsmoking women for this analysis. Recent SHS exposure as defined using urinary cotinine levels. A level of 0 ng/mL was used as the referent group (n=82). Those with cotinine levels less than and equal to 4 ng/mL were classified as low recent SHS exposure (n=42), and those with cotinine levels greater than 4 ng/mL were classified as high recent SHS exposure (n=36). Women with urinary cotinine values of 100 ng/mL or self-reported as a current smoker or that quit smoking less than a month ago were excluded from this analysis. Table 10 presents the characteristics of 160 nonsmoking women stratified by their amount of SHS exposure measured through urinary cotinine levels.

As shown in Table 10, nonsmoking women with highest recent SHS exposure (urinary cotinine levels greater than 4 ng/mL) were younger in age (P = 0.06), more likely to be obese (P = 0.04), more likely to have children (P = 0.002), less likely to exercise (P = 0.10), less likely to use MAR (P = 0.07), more likely to have a SA (P = 0.17), more likely to be slow acetylators (P = 0.072) and have less follow-up time (P =

0.03) compared to nonsmoking women with no recent SHS exposure (urinary cotinine level of 0 ng/mL). Descriptive statistics comparing urinary cotinine levels stratified by MAR can be found in the Appendix D (Table S3-4).

Table 10: Characteristics of Nonsmoking Women Stratified by Recent SHS Exposure Using Urinary Cotinine (N=160)

	Cotinine = 0 mg/dL N=82	**0 < Cotinine ≤ 4mg/dL N=42**	**4 < Cotinine ≤ 47 mg/dL N=36**	**P-value**
Age in years, median (Q_1-Q_3)	34.0 (30.00-37.75)	31.50 (27.25-35.75)	31.00 (26.00-35.25)	0.06
Age in years, n (%)				0.08
≤33	37(45.12)	26(61.90)	23(63.89)	
>33	45(54.88)	16(38.10)	13(36.11)	
BMI (kg/m^2), median (Q_1-Q_3)	27.18 (23.76-32.07)	31.49 (27.73-37.66)	31.14 (25.46-36.41)	0.003
BMI Category kg/m^2, n (%)				0.13
<25 (Underweight and Normal)	26(31.71)	7(16.67)	9(25.00)	
25 - 29.9 (Overweight)	27(32.93)	11(26.19)	8(22.22)	
≥ 30 (Obese)	29(35.37)	24(57.14)	19(52.78)	
BMI kg/m^2, n (%)				0.04
≤30	53(64.63)	18(42.86)	17(47.22)	
>30	29(35.37)	24(57.14)	19(52.78)	
Gravidity, n (%)				0.002
0	30(36.59)	29(69.05)	10(27.78)	
1	19(23.17)	7(16.67)	10(27.78)	
>=2	33(40.24)	6(14.29)	16(44.44)	
Regular Period, n (%)				0.26
No	25(30.49)	19(45.24)	12(33.33)	
Yes	57(69.51)	23(54.76)	24(66.67)	
Average Cycle Length (days), median (Q_1-Q_3)	28.00 (27.25-30.00)	28.00 (27.50-29.25)	29.00 (26.50-30.00)	0.85

Age of Menarche, in years, median (Q_1-Q_3)	13(12-14)	12(11-14)	12(12-14)	0.50
Previous STD, n (%)				0.91
No	52(63.41)	27(65.85)	22(61.11)	
Yes	30(36.59)	14(34.15)	14(38.89)	
Marital Status, n (%)				0.20
No	21(25.61)	9(21.43)	14(38.89)	
Yes	61(74.39)	33(78.57)	22(61.11)	
Routine Exercise, n (%)				0.10
No	27(34.18)	16(40.00)	19(55.88)	
Yes	52(65.82)	24(60.00)	15(44.12)	
Alcohol Use, n (%)				0.94
No	73(90.12)	37(90.24)	31(88.57)	
Yes	8(9.88)	4(9.76)	4(11.43)	
Caffeine Use, n (%)				0.75
No	45(54.88)	26(61.90)	21(58.33)	
Yes	37(45.12)	16(38.10)	15(41.67)	
Race, n (%)				0.33
Other	66(80.49)	30(71.43)	25(69.44)	
Black	16(19.51)	12(28.57)	11(30.56)	
AMH (ng/mL), median (Q_1-Q_3)	2.17 (0.86-3.95)	3.23 (1.09-5.56)	2.60 (1.59-5.61)	0.33
Length of Infertility years, median (Q_1-Q_3)	2.00 (1.00-4.00)	2.00 (1.31-4.25)	2.21 (1.00-5.81)	0.54
Treatment Cycles, n (%)				0.07
0	38(46.34)	23(54.76)	21(58.33)	
1	17(20.73)	7(16.67)	10(27.78)	
2	12(14.63)	4(9.52)	5(13.89)	
3 and more	15(18.29)	8(19.05)	0(0.00)	
NAT2 Acetylator Status, n (%)				0.07
Rapid	47(64.38)	23(60.53)	14(41.18)	
Slow	26(32.62)	15(39.47)	20(58.82)	
Pregnant, n (%)				0.82
No	49(59.76)	23(54.76)	22(61.11)	

Yes	33(40.24)	19(45.24)	14(38.89)	
Pregnancy Outcome, n (%)				0.17
Live Birth	21(72.41)	9(60.00)	5(41.67)	
SA	8(27.59)	6(40.00)	7(58.33)	
Total Follow-up Time (years), median (Q_1-Q_3)	8.00 (4.00-16.00)	5.00 (3.00-14.00)	5.00 (2.75-9.75)	0.03

3) Secondhand Smoke Exposure – Past Year's SHS Exposure (SSQ) among Nonsmokers

Self-reported SHS exposure in the past year was measured using two questions from the SSQ. Each question was scored never (1), rarely (2), often (3) and every day (4) and then summed. High SHS exposure was defined with a combined SSQ score ≥ 4 (n=57) and compared to low SHS exposure with a combined SSQ score ≤ 3 (n=112). Women with urinary cotinine values of 100 ng/mL or self-reported as a current smoker or that quit smoking less than a month ago were excluded from this analysis. Table 11 presents the characteristics of 169 nonsmoking women stratified by SHS exposure in the past year measured from the SSQ.

Nonsmoking women who self-reported high SHS exposure (combined SSQ score of ≥ 4) in the past year (n=57) were less likely to be married (P=0.01), more likely to be of African American race (P = 0.03), reported longer lengths of infertility (P = 0.02), were less likely to use MAR (P = 0.04) and less likely to conceive (P = 0.14), and were more likely to have a SA (P = 0.16), compared to nonsmoking women who self-reported low SHS exposure (combined SSQ score ≤ 3) in the past year. Descriptive statistics for

SHS exposure via the SSQ, stratified by MAR can be found in the Appendix D (Table S5-6).

Table 11: Characteristics of Nonsmoking Women Stratified by SHS Exposure in the Past Year Using the SSQ (N=169)

	No and Low exposure (score=2,3) N=112	Med and High exposure (score ≥4) N=57	P-value
Cotinine Levels, mg/dL median (Q_1-Q_3)	0.00 (0.00-1.00)	2.50 (0.00-10.40)	<0.001
Cotinine Categories, n (%)			<0.001
0 mg/dL	65(61.90)	17(30.91)	
<4 mg/dL	26(24.76)	11(20.00)	
>= 4 mg/dL	14(13.33)	27(49.09)	
Age in years median (Q_1-Q_3)	33.00 (29.00-37.00)	32.00 (27.00-35.00)	0.36
Age in years, n (%)			0.56
≤33	58(51.79)	33(57.89)	
>33	54(48.21)	24(42.11)	
BMI, kg/m2 median (Q_1-Q_3)	28.43 (24.81-32.39)	24.95 (24.95-37.25)	0.23
BMI Category, kg/m2, n (%)			0.65
<25 (Underweight and Normal)	30(26.79)	16(28.07)	
25 - 29.9 (Overweight)	35(31.25)	14(24.56)	
≥ 30 (Obese)	47(41.96)	27(47.37)	
BMI kg/m2, n (%)			0.52
≤30	65(58.04)	30(52.63)	
>30	47(41.96)	27(47.37)	
Gravidity, n (%)			0.90
0	48(42.86)	26(45.61)	
1	26(23.21)	12(21.05)	

>=2	38(33.93)	19(33.33)	
Regular Period, n (%)			0.51
No	36(32.14)	22(38.60)	
Yes	76(67.86)	35(61.40)	
Average Cycle Length, (days), median (Q_1-Q_3)	28.00 (28.00-30.00)	28.00 (27.00-23000)	0.82
Age of Menarche in years, median (Q_1-Q_3)	13.00 (12.00-14.00)	12.00 (12.00-13.75)	0.39
Previous STD, n (%)			0.79
No	72(64.86)	35(61.40)	
Yes	39(35.14)	22(38.60)	
Marital Status, n (%)			0.01
No	23(20.54)	23(40.35)	
Yes	89(79.46)	34(59.65)	
Routine Exercise, n (%)			0.21
No	40(37.38)	27(49.09)	
Yes	67(62.62)	28(50.91)	
Alcohol Use, n (%)			0.21
No	60(53.57)	37(64.91)	
Yes	52(46.43)	20(35.09)	
Caffeine Use, n (%)			0.97
No	22(19.82)	12(21.43)	
Yes	89(80.18)	44(78.57)	
Race, n (%)			0.03
Other	91(81.25)	37(64.91)	
Black	21(18.75)	20(35.09)	
AMH, ng/mL median (Q_1-Q_3)	2.46 (1.13-4.96)	2.51 (1.14-6.28)	0.62
Length of Infertility, in years, median (Q_1-Q_3)	1.75 (1.00-3.17)	3.00 (1.25-6.42)	0.02
Treatment Cycles, n (%)			0.04
0	50(44.64)	37(64.91)	
1	25(22.32)	10(17.54)	
2 and more	37(33.04)	10(17.54)	
NAT2 Acetylator Status, n (%)			0.83

Rapid	55(59.14)	29(55.77)	
Slow	38(40.86)	23(44.23)	
Pregnant, n (%)			0.14
No	62(55.36)	39(68.42)	
Yes	50(44.64)	18(31.58)	
Pregnancy Outcome, n (%)			0.16
Live Birth	29(70.73)	8(47.06)	
SA	12(29.27)	9(52.94)	
Total Follow-up Time, (years), median (Q_1-Q_3)	7.00(3.00-15.00)	5.00(3.00-13.00)	0.60

4) Combined Exposure – Active Smoking and SHS Exposure

The combined exposure variable compared nonsmokers with low SHS exposure in the past year (n=112) to nonsmokers with high SHS exposure (n-=57) and then to current smokers (n=54). The characteristics of 223 women using the combined effect exposure variable are shown in Table 12. Current smokers were more likely to have a previous STD (P = 0.12), less likely to be married (P<0.001), less likely to exercise (P = 0.07), more likely to be of African American race (P<0.001), less likely to use MAR (P<0.001), less likely to conceive (P = 0.07) and more likely to have a SA/miscarriage (P = 0.014), when compared nonsmokers with high SHS exposure (n=57) then to nonsmokers with low SHS exposure (n=112). Descriptive statistics for the combined exposure, stratified by MAR can be found in the Appendix D (Table S7-8).

Table 12: Characteristics of Women Using the Combined Effect Variable (N=223)

	Nonsmokers with low SHS N=112	Nonsmokers with high SHS N=57	Smokers N=54	P - value
Cotinine Levels, mg/dL, median (Q_1-Q_3)	0.00 (0.00-1.00)	2.50 (0.00-10.40)	100.00 (100.0-100.0)	< 0.001
Cotinine Categories, n (%)				
0 mg/dL	65(61.90)	17(30.91)	0(0.00)	< 0.001
<100 mg/dL	40(38.10)	38(69.09)	2(3.85)	
100mg/dL	0(0.00)	0(0.00)	50(96.15)	
Age in years, median (Q_1-Q_3)	33.00 (29.00-32.76)	32.00 (27.00-35.00)	32.50 (27.25-36.00)	0.51
Age in years, n (%)				0.59
≤33	58(51.79)	33(57.89)	32(59.26)	
>33	54(48.21)	24(42.11)	22(40.74)	
BMI, kg/m2, median (Q_1-Q_3)	28.43 (24.81-32.39)	29.04 (24.95-37.25)	30.36 (26.77-34.46)	0.21
BMI Category kg/m2, n (%)				0.53
<25 (Underweight and Normal)	30(26.79)	16(28.07)	11(20.37)	
25 - 29.9 (Overweight)	35(31.25)	14(24.56)	13(24.07)	
≥ 30 (Obese)	47(41.96)	27(47.37)	30(55.56)	
BMI kg/m2, n (%)				0.26
≤30	65(58.04)	30(52.63)	24(44.44)	
>30	47(41.96)	27(47.37)	30(55.56)	
Gravidity, n (%)				0.78
0	48(42.86)	26(45.61)	19(35.19)	
1	26(23.21)	12(21.05)	12(22.22)	
>=2	38(33.33)	19(33.33)	23(42.59)	
Regular Period, n (%)				0.70
No	36(32.14)	22(38.60)	18(33.33)	

Yes	76(67.86)	35(61.40)	36(66.67)	
Average Cycle Length, (days), median (Q_1-Q_3)	28.00 (28.00-30.00)	28.00 (27.00-30.00)	29.50 (28.00-30.00)	0.03
Age of Menarche, in years, median (Q_1-Q_3)	13.00 (12.00-14.00)	12.00 (12.00-13.75)	12.00 (11.00-13.00)	0.19
Previous STD, n (%)				0.12
No	72(64.86)	35(61.40)	25(48.08)	
Yes	39(35.14)	22(38.60)	27(51.92)	
Marital Status, n (%)				<0.0001
No	23(20.54)	23(40.35)	37(68.52)	
Yes	89(79.46)	34(59.65)	17(31.48)	
Routine Exercise, n (%)				0.07
No	40(37.38)	27(49.09)	28(56.00)	
Yes	67(62.62)	28(50.91)	22(44.00)	
Alcohol Use, n (%)				0.33
No	60(53.57)	37(64.91)	29(53.70)	
Yes	52(46.43)	20(35.09)	25(46.30)	
Caffeine Use, n (%)				0.68
No	22(19.82)	12(21.43)	8(15.09)	
Yes	89(80.18)	44(78.57)	45(84.91)	
Race, n (%)				<0.001
Other	91(81.25)	20(35.09)	27(50.00)	
Black	21(18.75)	37(64.91)	27(50.00)	
AMH, ng/mL median (Q_1-Q_3)	2.46 (1.13-4.96)	2.51 (1.14-6.28)	2.48 (1.52-5.66)	0.73
Length of Infertility, years, median (Q_1-Q_3)	1.75 (1.00-3.17)	3.00 (1.25-6.42)	2.50 (1.33-5.50)	0.02
Treatment Cycles,				<0.001

n (%)				
0	50(44.64)	37(64.91)	43(79.63)	
1	25(22.32)	10(17.54)	3(5.56)	
2 and more	37(33.04)	10(17.54)	8(14.81)	
NAT2 Acetylator Status, n (%)				0.47
Rapid	55(59.14)	29(55.77)	33(67.35)	
Slow	38(40.86)	23(44.23)	16(32.65)	
Pregnant, n (%)				0.07
No	62(55.36)	39(68.42)	39(72.22)	
Yes	50(44.64)	18(31.58)	15(27.78)	
Pregnancy Outcome, n (%)				0.02
Live Birth	29(70.73)	8(47.06)	4(28.57)	
SA	12(29.27)	9(52.94)	10(71.43)	
Total Follow-up Time, years, median (Q_1-Q_3)	7.00 (3.00-15.00)	5.00 (3.00-13.00)	7.00 (2.25-15.00)	0.88

Descriptive statistics comparing the characteristics of all 223 women stratified by MAR use is displayed in Table 13. Overall, MAR users since enrollment were significantly older (P = 0.04), less likely to be obese (P = 0.06), more likely to be married (P = 0.01), more likely to drink alcohol (P = 0.03), more likely to get pregnant (P <0.001), less likely to be a current smoker (P <0.001), and less likely to have high urinary cotinine levels (P <0.001), compared to women who did not use MAR since enrollment.

Table 13 : Characteristics of Women Stratified by MAR Use

		No MAR N = 130	MAR N = 93	P value
Age in years median (Q_1-Q_3)		31.50 (27.00-36.00)	34.00 (30.00-38.00)	0.04
Age in years, n(%)				0.06
	≤33	79(60.77)	44(47.31)	
	>33	51(39.23)	49(52.69)	
BMI (kg/m2)		30.45 (24.85-36.33)	28.53 (25.16-32.71)	0.10
BMI Category kg/m2, n(%)				0.04
	<25 (Underweight and Normal)	34(26.15)	23(24.73)	
	25 - 29.9 (Overweight)	28(21.54)	34(36.56)	
	≥ 30 (Obese)	68(52.31)	36(38.71)	
BMI kg/m2, n(%)				0.06
	≤30	62(47.69)	57(61.29)	
	>30	68(52.31)	36(38.71)	
Gravidity, n(%)				0.46
	0	51(39.23)	42(45.16)	
	1	28(21.54)	22(23.66)	
	≥2	51(39.23)	29(31.18)	
Regular Period, n(%)				0.53
	No	47(36.15)	29(31.18)	
	Yes	83(63.85)	64(68.82)	
Average Cycle Length (days) median (Q_1-Q_3)		28.50 (28.00-30.00)	29.00 (28.00-30.00)	0.87
Age of Menarche (in years) median (Q_1-Q_3)		12.00 (11.00-14.00)	13.00 (12.00-13.75)	0.62
Previous STD, n(%)				0.72
	No	75(58.59)	57(61.96)	
	Yes	53(41.41)	35(38.04)	
Marital Status, n(%)				0.01
	No	58(44.62)	25(26.88)	

Yes	72(55.38)	68(73.12)	
Routine exercise, n(%)			0.23
No	59(48.76)	36(39.56)	
Yes	62(51.24)	55(60.44)	
Alcohol Use, n(%)			0.03
No	82(63.08)	44(47.31)	
Yes	48(36.92)	49(51.69)	
Caffeine Use, n(%)			1.00
No	24(18.90)	18(19.35)	
Yes	103(81.10)	75(80.65)	
Race, n(%)			0.004
Black	50(38.46)	18(19.35)	
Other	80(61.54)	75(80.65)	
AMH (ng/mL) median (Q_1-Q_3)	2.54 (1.06-5.60)	2.40 (1.37-4.99)	0.78
Length of Infertility (in years) median (Q_1-Q_3)	2.00 (1.50-2.00)	1.50 (1.00-5.00)	0.02
NAT2 Acetylator Status, n(%)			0.82
Rapid	70(61.40)	47(58.75)	
Slow	44(38.60)	33(41.25)	
Pregnant, n(%)			<0.001
No	101(77.69)	39(41.94)	
Yes	29(22.31)	54(58.06)	
Pregnancy Outcome, n(%)			0.67
Live Birth	15(62.50)	26(54.17)	
SA	9(37.50)	22(45.83)	
Total Follow-up Time in years, median (Q_1-Q_3)	8.00 (3.00-15.00)	5.00 (3.00-12.00)	0.13
Cotinine Levels mg/dL, median (Q_1-Q_3)	4.75 (0.00-100.00)	0.20 (0.00-5.13)	<0.001
Cotinine mg/dL, n(%)			<0.001
0	38(30.64)	44(50.00)	
<100	46(37.10)	34(38.64)	
10	40(32.26)	10(11.36)	

Current Active Smoker, n(%)			<0.001
No	87(66.92)	82(88.17)	
Yes	43(33.08)	11(11.83)	
Self-reported SHS Exposure among Nonsmokers, n(%)			0.28
No/Low	93(71.54)	73(78.49)	
Intermediate/High	37(28.46)	20(21.51)	
Self-reported SHS Exposure among All, n(%)			0.05
No	24(24.74)	27(30.68)	
Low	30(30.93)	37(42.05)	
Intermediate	29(29.90)	20(22.73)	
High	14(14.43)	4(4.55)	

Specific Aim 1a: The Effect of Active Smoking on Fecundability

The association of active smoking (current and lifetime) on fecundability was modelled among three populations which included all follow-up time, MAR cycles and NC follow-up time. Full models were adjusted for MAR cycle, age, BMI, marital status, gravidity and race, as described in Table 8. Age and BMI were retained in all models, along with confounders that changed the effect of smoking by at least 10%. Dose-response relationships were assessed by treating the exposure variable ordinally and p-values and 95% confidence intervals were reported. The proportional hazards assumption was met for all variables in each model. The results for active smoking on fecundability are presented in Table 14.

Among all 223 women, current active smoking was not significantly associated with fecundability. Smokers had a 27% higher rate of fecundability compared to

nonsmokers, minimally adjusted for age, BMI and marital status (aFR: 1.27; 95% CI: 0.68, 2.38; p = 0.46). Compared to nonsmokers, women with 6 or more pack-years showed a 17% lower rate of fecundability (95% CI: 0.40, 1.70) and those with 1-5 pack-years had a 17% higher rate of fecundability (95% CI: 0.58, 2.38), after adjusting for age and BMI. The estimates and the test for trend, however, were also not statistically significant (p-trend = 0.83). The total number of cigarettes per day was not associated with fecundability, nor was a dose-response established (p-trend = 0.99).

The sub-population that analyzed MAR cycles only included 93 women. The full model included the same variables as the previous, but MAR type was included as the time-varying covariate instead of MAR cycle. The results among MAR cycles were similar to the all follow-up time models, in that current, active smoking was not significantly associated with fecundability. Smokers showed an insignificant 88% higher rate of fecundability (95% CI: 0.73, 4.85) compared to nonsmokers with adjustment for age, BMI and marital status. Among MAR cycles, women with 6 or more pack-years and 1-5 pack-years had a 20% (95% CI: 0.42, 3.46) and 31% higher rate of fecundability (95% CI: 0.65, 2.65) compared to women with no pack-years, respectively. The test for trend was not significant (p-trend = 0.44). Total number of cigarettes per day was not associated with fecundability, nor was a dose-response effect established (p-trend = 0.22).

The sub-population that analyzed NC follow-up time only included 212 women. The full models included age, BMI, gravidity, marital status and race. In this group, smoking was generally associated with a non-significant reduction in fecundability. Among NC follow-up time, current, active smokers showed a 22% reduction in fecundability (95% CI: 0.34, 1.79) compared to nonsmokers after adjusting for age, BMI

and marital status, but the estimate was not significant (p = 0.56). Women with NC follow-up time that had 6 or more pack-years and 1-5 pack-years had a 24% and 9% decrease in fecundability compared to women with no pack-years, respectively. The estimates were adjusted for age and BMI and were not statistically significant, along with the test for trend (p-trend=0.54). After adjusting for age, BMI and marital status, women who smoked a total of 10 or more cigarettes a day had a 33% (95% CI: 0.28, 1.58) reduction in fecundability and women who smoke 1 to 9 cigarettes a day had a 4% (95% CI: 0.32, 2.89) reduction in fecundability, compared to nonsmokers. The test for trend were also not statistically significant (p-trend = 0.37).

Table 14: Multivariable Cox Regression Models for Active Smoking (Current and Lifetime) on Fecundability among Three Populations

		FULL MODEL*		**MINIMALLY ADJUSTED MODEL[a]**	
	N (#events)	**Fecundability Ratio (95% CI)**	**P-value**	**Fecundability Ratio 95% CI**	**P-value**
		ALL FOLLOW-UP TIME			
Current Active Smoking					
Nonsmoker	169(68)	1.00 (Referent)		1.00 (Referent)	
Current active smoker	54(15)	1.26 (0.66, 2.41)	0.48	1.27[b] (0.68, 2.38)	0.46
Total Cigarettes Smoked per Day				**p-trend = 0.99**	
Nonsmokers	156(59)	1.00 (Referent)		1.00 (Referent)	
≤ 9	30(12)	1.08 (0.49, 2.36)	0.85	1.17 (0.58, 2.38)	0.66
≥ 10	37(12)	1.03 (0.52, 2.05)	0.92	0.83 (0.40, 1.70)	0.61
Pack-years				**p-trend = 0.83**	
Nonsmokers	156(59)	1.00 (Referent)		1.00 (Referent)	
≤ 5	37(15)	1.21 (0.60, 2.45)	0.59	1.17 (0.58, 2.38)	0.66
≥ 6	30(9)	0.85 (0.40, 1.78)	0.66	0.83 (0.40, 1.70)	0.61

MAR CYCLES					
Current Active Smoking					
Nonsmoker	82(41)	1.00 (Referent)		1.00 (Referent)	
Current active smoker	11(6)	1.97 (0.72, 5.37)	0.19	1.88[b] (0.73, 4.85)	0.19
Total Cigarettes Smoked per Day				**p-trend = 0.22**	
Nonsmokers	71(34)	1.00 (Referent)		1.00 (Referent)	
≤ 9	11(8)	1.11 (0.50, 2.45)	0.80	1.09[b] (0.52, 2.29)	0.82
≥ 10	11(5)	1.78 (0.75, 4.23)	0.19	1.87[b] (0.85, 4.11)	0.12
Pack-years				**p-trend = 0.44**	
Nonsmokers	71(34)	1.00 (Referent)		1.00 (Referent)	
≤ 5	13(10)	1.36 (0.63, 2.96)	0.44	1.31[b] (0.65, 2.65)	0.45
≥ 6	9(3)	1.09 (0.37, 3.19)	0.88	1.20[b] (0.42, 3.46)	0.73
NC FOLLOW-UP TIME					
Current Active Smoking					
Nonsmoker	160(27)	1.00 (Referent)		1.00 (Referent)	
Current active smoker	52(9)	0.73 (0.31, 1.68)	0.45	0.78[b] (0.34, 1.79)	0.56
Total Cigarettes Smoked per Day				**p-trend = 0.37**	
Nonsmokers	148(25)	1.00 (Referent)		1.00 (Referent)	
≤ 9	28(4)	0.91 (0.31, 2.68)	0.86	0.96[b] (0.32, 2.89)	0.94
≥ 10	36(7)	0.64 (0.26, 1.54)	0.32	0.67[b] (0.28, 1.58)	0.36
Pack-years				**p-trend = 0.54**	
Nonsmokers	148(25)	1.00 (Referent)		1.00 (Referent)	
≤ 5	36(5)	0.82 (0.31, 2.17)	0.68	0.91 (0.33, 2.48)	0.85
≥ 6	28(6)	0.65 (0.25, 1.69)	0.38	0.76 (0.31, 1.83)	0.54

* - Full models for NC follow-up time adjusted for Age, BMI, Gravidity, Marital Status and Race

Full models for MAR cycles adjusted for MAR Type, Age, BMI, Gravidity, Marital status and Race

Full models for All follow-up time models adjusted for MAR cycle, Age, BMI, Gravidity, Marital status and Race

a - Adjusted for Age and BMI

b - Adjusted for Age, BMI and Marital Status

Abbreviations: MAR - Medically Assisted Reproduction, NC - Natural Conception

Specific Aim 1b: The Effect of SHS Exposure on Fecundability among Nonsmokers

The association of SHS exposure (recent and past) on fecundability was modelled for the same three populations among nonsmokers: all follow-up time, MAR cycles, and NC follow-up time. Recent SHS exposure was measured using urinary cotinine levels and past SHS exposure using the SSQ. All models under this aim excluded current, active smokers. The proportional hazards assumption was met for all variables in the models. The results for SHS exposure (recent and past year) on fecundability are presented in Table 15.

In general, SHS exposure was not significantly associated with fecundability. Among all nonsmokers (n = 169), women with high SHS exposure in the past year reported in the SSQ had a 17% higher rate of fecundability (95% CI: 0.69, 1.99) compared to low SHS exposure, adjusted for age, BMI and marital status. However, the estimate did not reach statistical significance (p = 0.56). Similarly, nonsmoking women with high recent SHS exposure (urinary cotinine levels 5-99 mg/dL) (FR: 2.12; 95% CI:1.08, 4.15) and low recent SHS exposure (urinary cotinine levels from 1-4 mg/dL) (FR: 1.04; 95% CI: 0.57, 1.91) had a higher rate of fecundability than unexposed women (urinary cotinine levels = 0 mg/dL), adjusted for age and BMI. The test for trend with urinary cotinine was also not significant (p-trend = 0.08).

Among nonsmoking women with MAR cycles, the full models included the same covariates as the previous models, but MAR type was used as the time-varying covariate instead of MAR cycle. In these women, high SHS exposure from the past year showed an insignificant 45% increase in fecundability (95% CI: 0.74, 2.87) compared to low SHS exposure from the past year, adjusted for age, BMI and marital status. Nonsmoking

women with high urinary cotinine and low urinary cotinine had an 84% increase and 28% decrease in fecundability compared to unexposed women, respectively. These estimates were adjusted for age, BMI, gravidity, marital status and race; and were not statistically significant, along with the test for trend (p-trend = 0.59).

Among NC follow-up time, the full models were adjusted for age, BMI, gravidity, marital status and race. Nonsmoking women with high SHS exposure from the past year had a 4% insignificant reduction in fecundability adjusted for age and BMI (95% CI: 0.32, 1.51), compared to low SHS exposure from the past year. However, in the same population, nonsmoking women who had high and low levels urinary cotinine levels had a 36% (95% CI: 0.49, 3.74) and 22% (95% CI: 0.50, 3.01) higher rate of fecundability compared to unexposed women, respectively. The test for trend (p-trend = 0.53) was not significant.

Table 15: Multivariable Cox Regression Models for SHS Exposure (Recent and Past Year) and Fecundability among Nonsmokers

	N (#events)	FULL MODEL*		MINIMALLY ADJUSTED MODEL[a]	
		Fecundability Ratio (95% CI)	P-value	Fecundability Ratio (95% CI)	P-value
ALL FOLLOW-UP TIME					
SHS Exposure in the Past Year From SSQ					
Low	112(50)	1.00 (Referent)		1.00 (Referent)	
High	57(18)	1.33 (0.78, 2.28)	0.29	1.17[b] (0.69, 1.99)	0.56
Cotinine mg/dL				**p-trend = 0.08**	
0 mg/dL	82(33)	1.00 (Referent)		1.00 (Referent)	
≤ 4 mg/dL	42(19)	1.08 (0.57, 2.05)	0.81	1.04 (0.57, 1.91)	0.90

5 - 99 mg/dL	36(14)	2.31 (1.15, 4.64)	0.02	2.12 (1.08, 4.15)	0.03
MAR CYCLES					
SHS Exposure in the Past Year From SSQ					
Low	62(33)	1.00 (Referent)		1.00 (Referent)	
High	20(8)	1.51 (0.71, 3.19)	0.29	1.45[b] (0.74, 2.87)	0.28
Cotinine mg/dL				**p-trend = 0.59**	
0 mg/dL	43(20)	1.00 (Referent)		1.00 (Referent)	
≤ 4 mg/dL	8(3)	0.72 (0.26, 1.96)	0.52	0.72[c] (0.26, 1.96)	0.52
5 - 99 mg/dL	15(8)	1.84 (0.67, 5.03)	0.24	1.84[c] (0.67, 5.03)	0.24
NC FOLLOW-UP TIME					
SHS Exposure in the Past Year From SSQ					
Low	104(17)	1.00 (Referent)		1.00 (Referent)	
High	56(10)	1.01 (0.41, 2.46)	0.99	0.96[b] (0.43, 2.15)	0.92
Cotinine mg/dL				**p trend = 0.53**	
0 mg/dL	77(13)	1.00 (Referent)		1.00 (Referent)	
≤ 4 mg/dL	38(7)	1.41 (0.55, 3.62)	0.48	1.22 (0.50, 3.01)	0.66
5 - 99 mg/dL	35(6)	1.23 (0.36, 4.16)	0.74	1.36 (0.49, 3.74)	0.56

* - Full models for NC follow-up time adjusted for Age, BMI, Gravidity, Marital Status and Race

Full models for MAR cycles adjusted for MAR Type, Age, BMI, Gravidity, Marital status and Race

Full models for All follow-up time models adjusted for MAR cycle, Age, BMI, Gravidity, Marital status and Race

a - Models adjusted for Age and BMI

b - Models adjusted for Age, BMI and Marital status

c - Models adjusted for Age, BMI, Gravidity, Marital status and Race

Abbreviations: MAR - Medically Assisted Reproduction, NC - Natural Conception

Specific Aim 1c: The Effect of the Combined Exposure Effect on Fecundability

The association of the combined effect of current, active smoking and SHS exposure on fecundability was modelled for the same three populations: all follow-up time, MAR cycles, and NC follow-up time. The same set of covariates in the active smoking and SHS exposure models were used in the full models. The proportional hazards assumption was not violated for any variables. The results for combined effect of active smoking and SHS on fecundability are presented in Table 16.

Similar to the previous models, tobacco smoke exposure was not associated with fecundability in these models. In the MAR cycles, tobacco smoke exposure was associated with a non-significant increase in fecundability; whereas in the NC follow-up time, it was associated with a non-significant decrease in fecundability.

Among all women, current smokers and nonsmokers with high SHS had a 39% (95% CI: 0.70, 2.64) and 29% (95% CI: 0.75, 2.13) increase in fecundability compared to nonsmokers with low SHS exposure, respectively. The estimates were adjusted for age, BMI and marital status and were not significant, along with the trend test (p-trend = 0.27).

Among 93 women who contributed MAR cycles, it was found that current smokers (aFR: 2.08; 95% CI: 0.79, 5.49) and nonsmokers with high SHS exposure (aFR: 1.31; 95% CI: 0.63, 2.73) had increased fecundability compared to nonsmokers with low SHS exposure, adjusted for age, BMI and marital status. These estimates, along with the test for dose-response effect (p-trend = 0.12) were insignificant.

Among the 212 women that contributed NC follow-up time, current smokers and nonsmokers with high SHS exposure showed a 22% (95% CI: 0.32, 1.91) and 1% (95%

CI: 0.45, 2.17) reduction in fecundability compared to nonsmokers with low SHS exposure, respectively. The estimates were adjusted for age, BMI and marital status; the test for trend was not significant (p = 0.60).

Table 16: Multivariable Cox Regression Model for Combined Effect of Active Smoking and SHS Exposure on Fecundability among Three Populations

	N (#events)	FULL MODEL*		MINIMALLY ADJUSTED MODEL[a]	
		Fecundability Ratio (95% CI)	P-value	Fecundability Ratio (95% CI)	P-value
ALL FOLLOW-UP TIME					
Combined Effect				**p-trend = 0.27**	
NS w/low SHS	112(50)	1.00 (Referent)		1.00 (Referent)	
NS w/high SHS	57(18)	1.31 (0.78, 2.20)	0.30	1.29 (0.75, 2.13)	0.34
Current smokers	54(15)	1.39 (0.71, 2.75)	0.34	1.39 (0.70, 2.64)	0.33
MAR CYCLES					
Combined Effect				**p-trend = 0.12**	
NS w/low SHS	62(33)	1.00 (Referent)		1.00 (Referent)	
NS w/high SHS	20(8)	1.23 (0.59, 2.57)	0.59	1.31 (0.63, 2.73)	0.47
Current smokers	11(6)	2.10 (0.76, 5.77)	0.15	2.08 (0.79, 5.49)	0.14
NC FOLLOW-UP TIME					
Combined Effect				**p-trend = 0.60**	
NS w/low SHS	103(17)	1.00 (Referent)		1.00 (Referent)	
NS w/high SHS	57(10)	0.96 (0.40, 2.28)	0.93	0.99 (0.45, 2.17)	0.98
Current smokers	52(9)	0.71 (0.28, 1.81)	0.47	0.78 (0.32, 1.91)	0.58

* - Full models for NC follow-up time adjusted for Age, BMI, Gravidity, Marital Status and Race
Full models for MAR cycles adjusted for MAR Type, Age, BMI, Gravidity, Marital status and Race

Full models for All follow-up time models adjusted for MAR cycle, Age, BMI, Gravidity, Marital status and Race
Abbreviations: NS - Nonsmokers, MAR - Medically Assisted Reproduction, NC - Natural Conception
a - Models adjusted for Age, BMI and Marital status

Specific Aim 1d: Interaction of Current, Active Smoking and NAT2 Acetylator Status on Fecundability

Effect modification of NAT2 acetylator status on the effect of smoking on fecundability was examined by adding an interaction term into the models used from Aim 1a, among the three populations. NAT2 acetylator status compared slow and rapid acetylators. A total of 29 women did not have NAT2 acetylator status, either because they did not provide a urine sample or had issues with DNA amplification/contamination, reducing the total sample to 194. All full models were adjusted for age, BMI, gravidity, marital status, race and NAT2 acetylator status. No statistically significant interactions were detected among any of the populations (Table 17). In the NC follow-up time model, current smokers with a slow acetylation status had a 41% reduction in fecundability compared to nonsmoking women with rapid acetylation status. The estimate was adjusted for age and BMI but was not statistically significant (95% CI:0.11, 3.27). Among the MAR cycles only model, current smokers with a slow acetylation status had 6.85 times the increase in fecundability compared to nonsmoking women with rapid acetylation status. However, this association may have been due to the fact that there were only 3 women in the highest risk group and all 3 conceived.

Table 17: Interaction of Current Active Smoking and NAT2 Acetylator Status on Fecundability

NAT2 Status/ Smoking Exposure	N (#events)	FULL MODEL*		MINIMALLY ADJUSTED MODEL[a]	
		Fecundability Ratio (95% CI)	P-value for interaction	Fecundability Ratio (95% CI)	P-value for interaction
ALL FOLLOW-UP TIME					
Rapid/ Nonsmoker	84(38)	(Referent)	0.55	(Referent)	0.52
Rapid/ Smoker	33(9)	0.97 (0.45, 2.12)		1.28 (0.58, 2.82)	
Slow/ Nonsmoker	61(21)	0.73 (0.42, 1.27)		0.75 (0.44, 5.14)	
Slow/ Smoker	16(5)	1.42 (0.53, 3.80)		1.92 (0.70, 5.25)	
MAR CYCLES					
Rapid/ Nonsmoker	30(22)	(Referent)	0.007	(Referent)	0.04
Rapid/ Smoker	5(3)	1.09 (0.37, 3.23)		1.38 (0.34, 5.58)	
Slow/ Nonsmoker	21(13)	1.10 (0.49, 2.46)		1.10 (0.50, 2.39)	
Slow/ Smoker	3(3)	6.85 (2.72, 17.14)		8.22 (2.54, 26.74)	
NC FOLLOW-UP TIME					
Rapid/ Nonsmoker	54(16)	(Referent)	0.64	(Referent)	0.63
Rapid/ Smoker	28(6)	0.95 (0.37, 2.45)		0.89 (0.33, 2.38)	
Slow/ Nonsmoker	40(8)	0.82 (0.36, 1.87)		0.80 (0.34, 1.88)	
Slow/ Smoker	13(2)	0.59 (0.11, 3.27)		0.54 (0.09, 3.33)	

* - Full models for NC follow-up time adjusted for Age, BMI, Gravidity, Marital Status and Race

Full models for MAR cycles adjusted for MAR Type, Age, BMI, Gravidity, Marital status and Race

Full models for All follow-up time models adjusted for MAR cycle, Age, BMI, Gravidity, Marital status and Race
a – Models adjusted for age and BMI for all cycles

Specific Aim 2a (Active Smoking), Specific Aim 2b (SHS Exposure) and Specific Aim 2c (Combined Effect Exposure) on Probability of Spontaneous Abortion

The association of active smoking (current and lifetime) and SHS exposure (recent and past year) and the combined effect exposure on SA were assessed using multivariable logistic regression. It should be reiterated that all tobacco smoke exposures were assessed pre-conception. The main outcome was SA, which was compared to live birth. Current active smoking compared smokers to nonsmokers, and a dose-response effect was examined using total cigarettes smoked per day. Lifetime smoking exposure was assessed using pack-years. Recent SHS exposure was measured using urinary cotinine levels and SHS exposure in the past year was measured using the SSQ score. Full models were adjusted for MAR, age, BMI, gravidity, marital status, race and alcohol use. Age and BMI were retained in all minimally adjusted models, along with confounders that changed smoking estimates more than 10%. Dose-response relationships were assessed using a test for trend and p-values were reported. Results are displayed in Table 18.

In general, tobacco smoke exposure (both active smoking and secondhand) was associated with an increase in the odds of spontaneous abortion. Among the 72 women with known pregnancy outcome data, fully adjusted models had similar estimate effects to the minimally adjusted models. Current, active smokers (aOR:4.14; 95% CI:0.99,

21.25) had a marginally significant increase in the odds of SA compared to nonsmokers, adjusted for age, BMI and marital status. Women with 6 or more pack-years (aOR:1.77; 95% CI: 0.48, 6.66) and 1-5 pack-years (aOR: 3.18; 95% CI: 0.58, 20.00) had increased odds of SA compared to nonsmokers. These estimate were adjusted for age, BMI, gravidity and marital status, and the test for trend was not significant (p = 0.10). Women who smoked 10 or more cigarettes a day (aOR: 2.03; 95% CI: 0.43, 9.29) and women who smoked 9 or fewer cigarettes a day (aOR: 1.93; 95% CI: 0.47, 9.38) had an insignificant increase in odds of SA compared to nonsmokers. These estimates were adjusted for age, BMI, gravidity, marital status and alcohol use and also, no dose-response effect was found (p-trend = 0.28).

Nonsmoking women with high SHS exposure in the past year had a significant increase in the odds of SA (aOR: 4.30; 95% CI: 1.14, 19.06) compared to low SHS exposure in the past year, adjusted for age and BMI. Among nonsmokers, high levels (aOR: 5.77; 95% CI: 1.09, 38.65, p = 0.05) and low levels (aOR: 2.67; 95% CI: 0.52, 16.23) of urinary cotinine showed an increased odds of SA compared to unexposed women, adjusted for age, BMI, gravidity and race. The test for trend revealed a significant dose-response (p-trend = 0.04).

Using the combined effect of active smoking and SHS exposure, current smokers (aOR: 6.28; 95% CI: 1.31, 37.92) and nonsmokers with high SHS exposure (aOR:3.20; 95% CI: 0.87, 12.70) also had a significantly increased odds of SA compared to nonsmokers with low SHS exposure. These estimates were adjusted for age, BMI, gravidity and marital status; and produced a significant dose-response effect (p-trend = 0.02).

Table 18: Multivariable Logistic Regression Models of Smoking Exposures and Spontaneous Abortion among All Conceptions

	N (#events)	FULL MODEL*		MINIMALLY ADJUSTED MODEL	
		Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value
Current Active Smoking	**72**				
Nonsmoker	58(21)	1.00 (Referent)		1.00 (Referent)	
Current, Active Smoker	14(10)	3.94 (0.85, 24.07)	0.10	4.14[b] (0.99, 21.25)	0.06
Total Cigarettes Smoked per Day				**p-trend = 0.28**	
Nonsmokers	49(17)	1.00 (Referent)		1.00 (Referent)	
≤ 9	11(7)	1.92 (0.43, 9.26)	0.40	1.93[e] (0.43, 9.29)	0.39
≥ 10	12(7)	2.07 (0.47, 9.66)	0.34	2.03[e] (0.47, 9.38)	0.35
Pack-years				**p trend = 0.10**	
Nonsmokers	49(17)	1.00 (Referent)		1.00 (Referent)	
≤ 5	14(8)	1.62 (0.42, 6.45)	0.48	1.77[c] (0.48, 6.66)	0.39
≥ 6	9(6)	2.93 (0.52, 18.88)	0.23	3.18[c] (0.58, 20.0)	0.19
SHS Exposure in the Past Year •					
Low	41(12)	1.00 (Referent)		1.00 (Referent)	
High	17(7)	4.24 (1.05, 20.61)	0.05	4.30[a] (1.14, 19.06)	0.04
Cotinine mg/dL •				**p trend = 0.04**	
0 mg/dL	29(8)	1.00 (Referent)		1.00 (Referent)	
≤ 4 mg/dL	15(6)	2.58 (0.50, 15.82)	0.27	2.67[d] (0.52, 16.23)	0.25
5 - 99 mg/dL	12(7)	5.26 (0.98, 35.51)	0.06	5.77[d] (1.09, 38.65)	0.05
Combined Effect				**p trend = 0.019**	
NS w/Low SHS Exposure	41(12)	1.00 (Referent)		1.00 (Referent)	
NS w/High SHS Exposure	17(9)	3.17 (0.86, 12.58)	0.09	3.20[c] (0.87, 12.7)	0.09
Smokers	14(10)	6.56 (1.24, 46.54)	0.04	6.28[c] (1.31, 37.92)	0.03

• - Models exclude current, active smokers

* - Full models adjusted for MAR conception, Age, BMI, Gravidity, Marital status, Race and Alcohol use
a - Models adjusted for MAR conception, Age and BMI
b - Models adjusted for MAR conception, Age, BMI, Marital status
c - Models adjusted for MAR conception, Age, BMI, Marital status and Gravidity
d - Models adjusted for MAR conception, Age, BMI (continuous), Gravidity and Alcohol use
e - Models adjusted for MAR conception, Age, BMI, Gravidity, Marital status and Alcohol use
Abbreviations: NS - Nonsmoker

Specific Aim 2d: Interaction of Current, Active Smoking and NAT2 Acetylator Status on Spontaneous Abortion

Effect modification between current active smoking and NAT2 acetylator status on SA was examined by adding an interaction term into the models used from Aim 2a, among the three populations. NAT2 acetylator status was defined as rapid or slow. However, 10 participants from this analysis were missing NAT2 acetylator status, either because they did not provide a urine sample, or had issues with DNA amplification/contamination, making a final sample of 62. All models were adjusted for age, BMI, gravidity, marital status, race, alcohol use and NAT2 acetylator status. Results showed no statistically significant interaction (Table 19). Current smokers with a slow acetylator status had a 69% increase (95% CI: 0.08, 37.6) in the odds of SA compared to nonsmoking women with rapid acetylator status. The estimate was adjusted for age, BMI and MAR conception but was not statistically significant (P = 0.28). This analysis was not stratified by MAR use because the samples became unreasonably small after stratification.

Table 19: Interaction of Current Active Smoking and NAT2 Acetylator Status with Spontaneous Abortion

NAT2 Status/ Smoking Exposure	N	FULL MODEL*		MINIMALLY ADJUSTED MODEL[a]	
		Fecundability Ratio (95% CI)	P-value for Interaction	Fecundability Ratio (95% CI)	P-value for Interaction
ALL Conceptions (72)					
Rapid/ Nonsmoker	32(8)	(Referent)	0.28	(Referent)	0.14
Rapid/ Smoker	8(6)	9.36 (1.65, 79.5)		8.91 (0.92, 136.3)	
Slow/ Nonsmoker	17(12)	5.95 (1.61, 25.02)		9.07 (1.98, 55.39)	
Slow/ Smoker	5(4)	1.69 (0.08, 37.6)		0.55 (0.03, 11.56)	

a – Models adjusted for Age and BMI
b – Models adjusted for MAR conception, Age, BMI, Gravidity, Marital status, Race, Alcohol use and NAT2 Acetylator status

Specific Aim 3a: Assessment of Selection Bias Due to Loss to Follow-up

The full dataset including women that contributed no follow-up time (n=257) was divided into two groups. Group 1 contained women that were not asked permission to be followed up (n=95) and Group 2 contained those who were asked (n=162). The population was then each stratified by the type of follow-up they received: either no follow-up, follow-up through medical record only; or personal follow-up by phone, email or mailing address. The characteristics of women from Group 1 and Group 2 can be found in the Appendix D (Table S9-10).

Characteristics of women in Group 2 were stratified by the type of follow-up: no follow-up, medical record follow-up only, and personal follow-up only. No significant differences were found between medical record follow-up and personal follow-up; therefore, these groups were combined for subsequent analyses.. This left "any follow-up" to be compared with "no follow-up" (Table 20).

Women lost to follow-up were more likely to have a higher BMI (P = 0.15), more likely to be of African American race (P = 0.02), more likely to be a current smoker (P = 0.01), more likely to have higher urinary cotinine levels (P = 0.004) and more likely to report higher SHS exposure in the past year (P = 0.09).

Table 20: Characteristics of Women Stratified by Follow-up Type(None vs. Any) (N=257)

	Lost to Follow-up N=28	**Medical Record or Personal N=229**	**P-value**
Age in years, median (Q_1-Q_3)	30.00 (25.50-35.00)	33.00 (28.00-37.00)	0.21
Age in years, n (%)			0.36
≤33	18(66.67)	127(55.46)	
>33	9(33.33)	102(44.54)	
BMI, kg/m2 median (Q_1-Q_3)	31.32 (24.43-42.54)	29.04 (24.82-34.85)	0.15
BMI Category, kg/m2, n (%)			0.70
<25 (Underweight and Normal)	7(26.92)	61(26.64)	
25 - 29.9 (Overweight)	5(19.23)	63(27.51)	
≥ 30 (Obese)	14(53.85)	105(45.85)	
BMI kg/m2, n (%)			0.57
≤30	12(66.67)	124(55.46)	

>30	14(33.33)	105(44.54)	
Gravidity, n (%)			0.26
0	7(25.93)	97(42.36)	
1	8(29.63)	51(22.27)	
>=2	12(44.44)	81(35.37)	
Regular Period, n (%)			0.11
No	14(51.85)	78(34.06)	
Yes	13(48.15)	151(65.94)	
Average Cycle Length, days, median (Q_1-Q_3)	28.00 (27.50-29.50)	29.00 (28.00-30.00)	0.36
Age of Menarche in years, median (Q_1-Q_3)	12.00 (11.00-13.00)	12.00 (11.00-14.00)	0.11
Previous STD, n (%)			0.78
No	17(65.30)	136(60.44)	
Yes	9(34.62)	89(39.56)	
Marital Status, n (%)			0.76
No	11(42.31)	85(37.12)	
Yes	15(57.69)	144(62.88)	
Routine Exercise, n (%)			0.37
No	13(56.52)	96(44.24)	
Yes	10(43.48)	121(55.76)	
Alcohol Use, n (%)			0.93
No	16(59.26)	129(56.33)	
Yes	11(40.74)	100(43.67)	
Caffeine Use, n (%)			0.43
No	3(11.54)	45(19.91)	
Yes	23(88.46)	181(80.09)	
Race, n (%)			0.02
Other	12(44.44)	158(69.00)	
Black	15(55.56)	71(31.00)	
AMH (ng/mL), median (Q_1-Q_3)	2.03 (0.76-4.82)	2.49 (1.30-5.03)	0.49

Length of Infertility, years median (Q_1-Q_3)	1.75 (0.79-4.00)	2.00 (0.92-4.25)	0.82
NAT2 Acetylator Status, n (%)			1.00
Rapid	15(60.00)	119(60.41)	
Slow	10(40.00)	78(39.59)	
Cotinine (mg/dL), median (Q_1-Q_3)	100.00 (2.25-100.00)	1.50 (0.00-38.00)	0.004
Cotinine mg/dL, n (%)			0.01
0	5(18.52)	84(38.71)	
<100	8(29.63)	81(37.33)	
100	14(51.85)	52(23.96)	
Current Active Smoker, n (%)			
No	14(50.00)	173(75.55)	0.01
Yes	14(50.00)	56(24.45)	
Self-reported SHS Exposure, n (%)			0.09
Low	9(32.14)	117(51.09)	
High	19(67.86)	112(48.91)	

Specific Aim 3b: Inverse Probability Weighting

Table 21 shows the weighted ORs and weighted FRs estimates adjusting for confounding and selection bias due to loss to follow-up, separately and jointly for conception as a dichotomous variable and fecundability. Results showed that there was little difference between adjusting for confounding only and adjusting for both confounding and selection bias due to loss of follow-up in all models. This may suggest that the results are not biased to loss to follow-up. These estimates were also compared to the estimates from the original analysis (Table 14). The original results from Table 14 showed an adjusted effect estimate in the opposite (anticipated) direction (aFR=1.26 95%

CI 0.66, 2.41); however, compared to the effect estimate from Table 21 that was adjusted for both confounding and selection bias due to loss of follow-up (aFR=0.87 95% CI 0.45, 1.68), the effect was in the anticipated direction. Overall, the conclusions remained consistent with no association between current smoking and fecundability.

Table 21: Weighted Estimates Using Inverse Probability Weighting

Model	Estimate	95% CI
Logistic Regression - Conception (All Women)	OR	
Unadjusted and Unweighted	0.57	0.29, 1.10
Adjusted for Confounding Only	0.99[a]	0.79, 1.23
Adjusting for Both Confounding and Selection Bias Due to Loss of Follow-up	0.98	0.80, 1.21
Logistic Regression - Conception (NC Follow-up Months)	OR	
Unadjusted and Unweighted	0.74	0.30, 1.69
Adjusted for Confounding Only	0.89[b]	0.77, 1.03
Adjusting for Both Confounding and Selection Bias Due to Loss of Follow-up	0.90	0.78, 1.05
Survival Analysis - Fecundability (All Follow-up Time)	FR	
Unadjusted and Unweighted	0.69	0.38, 1.23
Adjusted for Confounding Only	0.90[a]	0.46, 1.76
Adjusting for Both Confounding and Selection Bias Due to Loss of Follow-up	0.87	0.45, 1.68
Survival Analysis - Fecundability (NC Follow-up Months)	FR	
Unadjusted and Unweighted	0.95	0.44, 2.05
Adjusted for Confounding Only	0.70[b]	0.29, 1.70
Adjusting for Both Confounding and Selection Bias Due to Loss of Follow-up	0.73	0.31, 1.74

a - Adjusted for MAR cycle, Age, BMI, Gravidity, Marital status and Race
b - Adjusted for Age, BMI, Gravidity, Marital status and Race
Abbreviation: MAR - Medically Assisted Reproduction, NC- Natural Conception

VI. DISCUSSION

This preconception cohort study was done to examine the association of tobacco smoke exposures on fertility and fertility-related outcomes, among women of reproductive age seeking fertility counselling and treatment. Overall, smoking and SHS exposure were not appreciably associated with fecundability, but the directions of associations were opposite for women who used MAR vs. those who conceived naturally. Smoking and high SHS exposure were associated with increased odds of spontaneous abortion among women who conceived.

Active Smoking and Fecundability

Although no significant associations between tobacco smoke exposure and fecundability were observed in this study, the difference in the direction of association in those using MAR techniques vs. those who conceived naturally indicates that the effects of smoking in these two groups may be fundamentally different. Different biological mechanisms may be at play, which are discussed in more detail below. Additionally, the population who used MAR was different in that they were significantly older, less likely to be obese, more likely to be married, less likely to be a current smoker, and had lower urinary cotinine levels. Some of these factors may be modifying or attenuating the effect of smoking on the probability of conception using MAR. For example, the women using MAR may be 'healthier' in terms of their ability to conceive, due their lower BMI and

lower stress, because of a presumably better support system. Women using MAR were more likely to be married, which can act as a proxy for sexual intercourse frequency, emotional support or the seriousness of intention to become pregnant. These factors may directly or indirectly modify the effect of smoking on conception.

Our results did not show an effect of active smoking on probability of conception using MAR, which is consistent with other studies (178–181). However, there have been many studies that show associations with smoking and lower success among MAR, specifically using IVF (131,132,182,183). A recent meta-analysis in 2018, examined 26 studies with samples ranging from 40 to 834, found a significant overall association between active smoking on ART outcomes (Pooled OR: 0.53, 95%CI: 0.41, 0.68). Associations remained significant when the analysis was restricted to IVF cycles (16 studies, Pooled OR:0.52, 95%CI:0.39, 0.68). While, the authors did report a moderate amount of heterogeneity between studies, and heavy reliance on self-reported exposure data, there is strong evidence to suggest an association between smoking and conception among women using MAR. Our results are still consistent with a possible harmful effect; inference is difficult due to the small number of smokers (11) who ever used MAR, leading to wide confidence intervals.

For natural conceptions, there is a plethora of studies supporting the association of active smoking with longer time to conception and increased risk of infertility (38,125,127,130). However, the magnitude of the associations is not overwhelming, with the majority of studies producing weak effect sizes. Few studies have found significant dose-response effects for female cigarette use with infertility and fecundability (38,125,128). Curtis and colleagues found a significant dose-response effect among

heavier smokers when examining the effect of smoking on fecundability. Women smoking 11–20 cigarettes (aFR:0.87; 95%CI: 0.77, 0.99) and more than 20 cigarettes per day (aFR:0.74; 95%CI: 0.59, 0.92) had reduced fecundability (38). Wesselink and colleagues found similar weak associations and noted that the dose-response effect on fecundability may only be among heavy smokers (135). Other studies also found associations at higher intensities (25,126,184). Our study also found stronger reductions in fecundability with increasing smoking intensity and cumulative lifetime exposure. Although approximately 25% of our study population were active smokers, consistent with what is seen in Kentucky (92), we had few heavy smokers, and our estimates were not statistically significant. Overall, the evidence suggests that the effect of smoking on fecundability may be limited to the heavy smokers.

Secondhand Smoke Exposure and Fecundability

Overall, our results showed no effect of recent or past year's SHS exposure on fecundability among nonsmokers. The difference in the direction of association between women using MAR and women who conceived naturally can be explained similarly to the active smoking exposure. Within this population, nonsmoking women using MAR were significantly different, in that they had characteristics that have been shown to help increase fertility. These nonsmoking women using MAR were less likely to be obese, more likely to be married and had lower urinary cotinine levels, meaning they are healthier in some ways than women who did not use MAR, and had lower levels of exposure

For women who conceived naturally, a small but insignificant reduction in fecundability was reported for high SHS exposure compared to low SHS exposure. These results are consistent with the previous literature showing either weak associations (122,127) or no effect (126,139,140). Authors Radin and Wesselink both used prospective cohort studies to examine self-reported SHS exposure on fecundability with weak, insignificant findings (aFR: 0.92; 95% CI: 0.83,1.03) and (aFR: 0.93; 95%CI: 0.70,1.25), respectively(126,135). Benedict and colleagues retrospectively analyzed a cohort study among verified nonsmoking women undergoing IVF treatment and found that women with follicular fluid cotinine concentrations between 1.11 and 10 ng/ml had increased risk of implantation failure (aRR: 1.17;95%CI: 1.20,1.92) (122). Sapra and associates used prospective cohort study to examine heavy metals and serum cotinine concentrations on TTC. Authors found a significant association for serum cotinine levels greater than 10 ng/mL and longer TTC in females (aFR:0.64; 95%CI:0.41,.0.98) (127). However, the association was greatly attenuated after adjusting for cadmium levels, suggesting that cadmium may be responsible for some of the association between tobacco use and longer TTC.

There are several proposed mechanisms to explain how tobacco smoke exposure may affect fecundability. Studies have shown tobacco smoke toxins accumulate within follicular fluid and surrounding tissues (106,110). The harmful substances within tobacco smoke can affect gametogenesis, which can increase the risk of aneuploidies within embryos and affect folliculogenesis by lowering ovarian reserve through impaired follicle growth or development of oocytes (107,114). Some of the mechanisms include meiosis

impairment, abnormal intercellular crosstalk, DNA damage, high levels of oxidative stress, and activation of cell death pathways (107,108,110,114).

Heavy metals and toxins in tobacco smoke promote oxidative stress which can lead to deleterious effects on cell membranes, DNA and proteins (107,110). DNA damage can then lead to chromosomal abnormalities which in turn adversely affect fertilization (114). Cadmium and benzo(A)pyrene, which are commonly found in tobacco smoke, can also impair steroidogenesis, resulting in hormonal imbalances that can affect both fertilization and implantation (107,108). Nicotine is a well-known strong vasoconstrictor that can impair blood flow to the uterus. The structural function within the fallopian tubes, uterus and cervix is also compromised, as the toxins act as adhesions to lower ciliary cell activity, impair smooth muscle contraction and lower oocyte retrieval rates (108,111).

This study examined active smoking and SHS exposure from preconception in both natural and assisted conception, which allowed for detailed biological mechanisms to be hypothesized. Among natural conceptions, the observed effect of tobacco smoke exposures on reduced fecundability supported the mechanisms described earlier. However, for women using MAR, fecundability may not be affected by tobacco smoke exposure after OI or oocyte retrieval for IVF. This suggests that the toxins from cigarette smoke may only affect fecundability via folliculogenesis through impaired follicle growth or development of oocytes and steroidogenesis through hormonal imbalances that impair ovulation. However, after OI and oocyte retrieval for IVF-ET, smoking may not affect to a large extent other aspects such as implantation and/or development of the trophoblast.

This study is the first to analyze effect modification by NAT2 acetylator status on active smoking and fecundability among a clinical population. While no statistically significant interactions were detected among any of the populations, results may suggest lower fecundability for current smokers who are slow NAT2 acetylators for natural conceptions. However, the lack of significant associations may have been due to small sample sizes among subgroups of smokers within this study. One other study that found similar results examined the interaction of NAT2 and current smoking on fecundability among women office workers. They found significantly reduced fecundability among currents smokers with a slow acetylation status (15). The possible mechanism of effect modification may be through polycyclic aromatic hydrocarbons and heterocyclic amines, that are known constituents of tobacco smoke and are also metabolized by NAT2. Slow acetylators may experience greater accumulation of these constituents within the ovarian tissue and follicular fluid inducing higher levels of ovarian toxicity. Fecundability may be also affect by several mechanisms, including increased oxidative stress and DNA damage.

Active Smoking and Spontaneous Abortion

Among 72 conceptions with documented pregnancy outcomes, a marginally significant association between current active smoking and SA was observed. Results also showed increasing odds of SA with increasing intensity of smoking and cumulative lifetime exposure. However, no significant dose-response effect was established for either. Our results are consistent with Nielsen and colleagues, who also used preconception smoking exposure on SA in a nested case-control study. However, their

results showed a significant dose-response effect for every 5 cigarettes smoker per day (aOR: 1.20; 95%CI: 1.04,1.39) (144). Another recent case-control study also found a significant dose-response effect but for maternal smoking ≥20 cigarettes a day and SA (aOR: 2.39; 95%CI:1.26, 4.25) (185). While, there are a few studies that found significant dose-response effects, other studies reported no significant effect for smoking intensity and SA (143,186).

Studies among women undergoing ART have shown an association between maternal smoking and adverse ART outcomes, particularly SA. Budani and colleagues conducted a meta-analysis using 8 studies and found significantly increased odds of SA per clinical pregnancy rate and maternal smoking (Pooled OR: 2.22; 95%CI: 1.10,4.48). However, the authors did report a substantial amount of heterogeneity between the studies. The majority of studies within the literature used self-reported information about smoking, which can result in misclassification as women can either under-report their exposures or change their behaviors throughout the pregnancy. Variation in the accuracy of smoking exposures may explain some of the heterogeneity across studies. In our study, we verified recent smoking using urinary cotinine; however, we did not ask or verify whether the women continued to smoke during pregnancy. In addition, we were unable to stratify the SA analysis by MAR, due to small numbers. Nonetheless, our findings are consistent with the previous studies for both medically assisted and natural conceptions and demonstrate a harmful effect of smoking on ongoing conceptions, leading to spontaneous abortion.

The rate of spontaneous abortion in this study (43%) was noticeably higher than in the general population, commonly estimated to be 15-20%. However, these

pregnancies were closely followed, allowing for detection of early losses that might usually go unnoticed, especially for women who use MAR. Preconception tobacco smoke exposure captures a critical exposure period that can help elucidate potential biological mechanisms. It may be that through its proximal effects on the uterine environment, preconception smoking is more likely to cause early pregnancy losses (loss of 'biochemical pregnancies'), as opposed to later, clinical pregnancy losses. More studies examining preconception smoking exposures on both planned natural conceptions and MAR/ART outcomes are needed to understand the biological mechanisms of this critical exposure period.

Secondhand Smoke Exposure and Spontaneous Abortion

Among verified nonsmokers, high SHS exposure in the past year was significantly associated with increased odds of SA, with a significant dose-response effect for preconception urinary cotinine. The combined effect exposure also showed increased odds of SA for nonsmokers with high SHS exposure and even greater increased odds of SA for current smokers compared to nonsmokers with low SHS exposure, supporting the association with a significant test for trend.

It is possible that the effect of preconception maternal SHS exposure may be confounded by paternal smoking, whereby the observed effect of maternal SHS on SA is actually mediated by damaged sperm from the father. We investigated this possibility. We did not find evidence that the association of preconception maternal SHS was confounded by paternal smoking; of the women whose spouses smoked, the spouses were

not diagnosed with male factor infertility. This suggests that the estimate was not biased by confounding from spousal smoking or male factor infertility.

Several previous studies examined maternal exposure to SHS on SA found significant associations; however, there is lack of studies examining preconception SHS exposures on pregnancy outcomes. Many of the studies that found significant effects for maternal smoking on SA used weaker study designs and relied on unverified self-reported exposure data (145,149,150), likely attenuating any associations. One case-control study used plasma cotinine levels to examine SHS exposure on SA. The authors found a significant association for plasma cotinine levels between 0.1 and 15 ng/mL and increased odds of SA (aOR:1.67; 95%CI: 1.17,2.38) (151). There are, however, several studies that reported no association between SHS exposure during pregnancy and SA (139,140,152). Pineles and colleagues conducted a meta-analysis examining SHS exposure during pregnancy and SA using 17 studies. Researchers found no significant association between SHS exposure during pregnancy and SA (148). Sensitivity analyses retained insignificant estimates when studies among nonsmokers only were used. This study may have been able to detect small effects due to the sensitive measures of SHS from both urinary cotinine and a questionnaire; the ability to adjust for more relevant confounders than previous studies; and the ability to detect very early pregnancy losses.

It is difficult to make inferences regarding whether NAT2 acetylator status modified the effect of smoking on SA, because there were very few women in some of the subgroups. For example, there were only 3 smokers who were slow acetylators and all 3 conceived.

Several mechanisms by which tobacco smoke exposure affects spontaneous abortions have been hypothesized. These include DNA damage, apoptosis, impairment of tissue development, or disruptions within cell division and other processes (75,79). Aneuploidies can also form from DNA damage and increase the odds of chromosomal abnormalities which is the most common cause of SA (10,79). Cadmium and nicotine directly constrict blow flow to the uterus, impairing development of the placenta and consequently resulting in EPL (108). Other mechanisms include impairment of trophoblast growth, impairment of adhesions cells for embryo post-implantation and apoptosis of placental cells (108,111). A detailed exploration of tobacco smoke exposures during different critical periods of development will be important in elucidating potential biological mechanisms and much needed to infer causality.

This study examined active smoking and SHS exposure on SA within a population that is composed of infertile or subfertile women/couples. The majority of SA occurred among women who conceived using MAR (64.5%). Some potential mechanisms behind tobacco smoke exposure and SA among MAR users include accumulation of cotinine or other harmful toxins within the uterus, affecting processes after implantation such as restricting blood flow to the uterus and impairing placental development.

Strengths and Limitations

This study has several strengths. Firstly, its prospective cohort design allowed for assessment of preconception tobacco smoke exposures; and prospectively following the subjects from enrollment to pregnancy and pregnancy outcomes establishes temporality,

boosting the evidence for a causal association. Conception and pregnancy outcome data were either extracted from medical records in duplicate which increased their accuracy, or self-reported by women at follow-up intervals. Women who gave permission were personally contacted in approximately 6-month intervals after their enrollment to collect data. This allowed an ample amount of time for pregnancy, but short enough to minimize recall bias. Also, using a prospective cohort allows for time to conception analysis and adjustment for time-varying exposures and confounders. The analytic approach allowed for use of time-to-event data, as opposed to simply modeling the probability of a dichotomous outcome. Survival analyses therefore yield ratio estimates that are more robust and meaningful, compared to binary regression such as logistic regression.

A second strength of this study includes the high retention rate. Out of all 257 women, 229 (89.1%) were successfully followed up, either through information extracted from medical records and/or through personal re-contact if they gave permission. Female smoking was found to be related to attrition in that current smokers were more likely to be lost to follow-up. If the smokers who were lost to follow-up had a lower chance of conceiving than the smokers who remained in the study, the probability of conception among the smokers would be overestimated. However, after investigating the potential effects of selection bias due to loss of follow-up using IPW, the results showed little difference between adjusting for confounding only and adjusting for both confounding and selection bias due to loss of follow-up for both outcomes. This may suggest that the results are not biased due to loss to follow-up.

A third important strength of the study is the use of an objective biomarker to verify smoking status. As shown in this study, women tend to under-report their smoking

behaviors, which can cause misclassification and may cause bias towards the null, as it adds smokers to a presumably non-smoking group. In this study, 16 women reported being a nonsmoker or a former smoker, but their high cotinine levels indicated that they were currently smoking (>100 ng/mL). These women were grouped with verified current smokers based on their urinary cotinine levels at the time of enrollment.

Lastly, to the best of our knowledge, this is the only study to explore effect modification by NAT2 acetylator status on the relationship between smoking and fecundability, as well as spontaneous abortion in a clinical population. Investigating possible gene-environmental interactions can help to uncover potential biological mechanisms and also can help to explain heterogeneity in results of prior studies.

There are a few limitations that should be acknowledged. Firstly, external validity may be limited, given the specific clinical population used for this study. The results may not be generalizable to the public, as they may have significantly different characteristics compared women seeking fertility care. Secondly, the results were limited by the small sample size of heavy/intense smokers (≥10 cigarettes per day, n=37). Also, after stratifying the results for fecundability by MAR cycles only (n=70) and NC follow-up time only (n=153), there were even smaller samples within sub-groups, especially the MAR-cycles-only models. The analysis for SA was also a small subset of the original population (n=72 conceptions). This reduced the power to detect any possible interactions with NAT2 acetylator status on smoking with fecundability and SA. Small sample sizes within sub-groups affected the precision of the estimates, resulting in wide confidence intervals. Furthermore, given the small numbers there is a possibility that the associations

for tobacco smoke exposure on SA reported were due to chance, though this association is biologically plausible and has other support in the literature.

Preconception smoking is a critical exposure period that has not been fully explored especially with regard to SA. However, most women change or under-report their smoking behaviors when they become pregnant. Given that smoking was measured at one point in this study, it is not clear whether the effect of preconception smoking on SA is truly a lasting result of smoking prior to pregnancy, or if the effect is seen because the women kept smoking during pregnancy.

A fourth limitation of the study includes the restriction to women planning a pregnancy. Women seeking fertility care and treatment, with the intention of conceiving were eligible for the study. Theoretically, less fertile smokers would have been enrolled, given that smokers are more likely to have unplanned pregnancies either because of lack of contraceptive use or risky behaviors.

There is also the possibility of residual confounding from both measured and unmeasured confounders. To create a parsimonious model, many variables were treated continuously or simply dichotomized. The estimates for tobacco smoke exposure and SA were larger compared to past studies. This may reflect residual confounding from unmeasured variables. While the results were likely not confounded by spousal smoking or male factor infertility, there may have been other unmeasured confounders; high secondhand smoke exposure may reflect other adverse environmental exposures, or unhealthy social or behavioral exposures.

The rate of SA was significantly higher than expected at 43%. This is likely because it included early biochemical pregnancy losses. Information was not collected to separate early from late SA, which could have provided more insight into mechanism.

Time to conception was measured in months and not by menstrual cycles. Women with irregular menstrual cycles which is common among anovulatory infertility may not ovulate every month. Hence, using months may have overestimated time to conception and not accurately reflect cycles-at-risk.

Suggestions for Future Research

The 2020 U.S. Surgeon General report is still stating that there is insufficient evidence to infer an association between tobacco smoke exposure and SA because of many methodological concerns. Future studies should consider using more large-scale, longitudinal prospective studies to help infer causality and biomarkers to validate self-reported exposures. Dose-response effects and measuring tobacco smoke exposures at critical exposure periods and developmental windows, and at multiple intervals can help to fully capture the effects and elucidate biological mechanisms. Additionally, many of the studies that examined MAR procedures only took IVF into account; however, more research is needed to address whether the effects of smoking are observed across all treatment procedures including OI, IUI and FET.

When examining natural follow-up time, ovulation kits should be used so that the number of cycles of follow-up can be accurately determined. Careful consideration should also be taken when adjusting for all important confounders and examining potential effect modifiers, such as genetic variants and types of infertility diagnosis.

Information on paternal smoking should be collected. Lastly, cohort studies study should be designed to maximize retention and reduce potential selection bias and appropriate statistical analyses should also be done to assess and adjust for bias, such as selection and attrition bias, before reporting estimates that may reflect spurious associations.

Conclusion

Infertility and adverse pregnancy outcomes remain growing public health problems. There are several studies supporting the association of active smoking on lowered fecundability, increased infertility, and increased risk of adverse pregnancy outcomes. However, according to the latest U.S. Surgeon General's report, the literature is still insufficient with regard to SHS exposures. This study used a prospective cohort study design, along with objective markers of exposure assessment, adjusted for potential biases and explored a gene-environment interaction, to further clarify the effect of active smoking on both assisted and natural conceptions, and added to the small body of literature regarding the effect of SHS exposure on fecundability and SA.

Results from this study did not show an association for active smoking or SHS exposure on fecundability, but there was suggestive evidence that the effect of active smoking may be more pronounced among slow acetylators. Significant associations were found for preconception active smoking and SHS exposure with a significant dose-response effect for urinary cotinine on SA. These findings suggest that current smokers and nonsmokers with high levels of SHS exposure may have increased risk of adverse birth outcomes. Data from this dissertation can ultimately be used to support public policies that discourage smoking and limit the public's exposure to secondhand smoke.

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APPENDIX A - LOUSSI Supplemental Smoking Questionnaire

LOUSSI SUPPLEMENTAL SMOKING QUESTIONNAIRE
LIFETIME EXPOSURE TO TOBACCO SMOKE
INSTRUCTIONS

Dear Participant:
Thank you for taking the time to answer this questionnaire. This form should take 5 to 10 minutes to complete. Your answers will be used for research purposes only. Your doctor will not know the answers you have marked. Information gained from this study will help scientists and doctors understand the effect of smoking and secondhand smoke on fertility.

This packet contains three questionnaires.
Please choose ONE questionnaire to complete, depending on whether you are a current smoker, former smoker, or nonsmoker. Leave the other two questionnaires blank.

CURRENT SMOKERS: Please answer Questionnaire 1. You are considered a current smoker if you currently smoke at least 1 cigarette/week, or if you recently quit (less than 1 month ago)
FORMER SMOKERS: Please answer Questionnaire 2. You are considered a former smoker if you quit more than 1 month ago and used to smoke at least 1 cigarette/week.
NONSMOKERS: Please answer Questionnaire 3. You are considered a nonsmoker if you have never smoked more than 1 cigarette/week.

Please place questionnaire in the envelope provided and return it to the study personnel or place it inside the designated LOUSSI Study dropbox when you are finished.

Thank you very much for your time!

QUESTIONNAIRE 1 (FOR CURRENT SMOKERS)

Answer this questionnaire if you currently smoke at least 1 cigarette/week, or if you quit smoking less than 1 month ago.

Smoking History

1. How many years have you been smoking (at least 1 cigarette/week)? _____ years

   (If you have smoked on and off, please add up the total number of years you smoked.)

2. On the average, how many cigarettes (or packs) do you now smoke per day?

   ___ cigarettes/day or ____ packs/day

   (If you quit less than 1 month ago, please indicate how much you used to smoke before you quit.)

Secondhand Smoke

3. Think about your home **when you were growing up (less than 18 years old).** On average, how often were you exposed to secondhand smoke inside your home? (For example, how often did your parents, guardians, or siblings smoke inside the home?)

   Never | Rarely (less than once/week) | Often (1-6 days/week) | Every day

4. Think about your home **in the past year**. How often have you been exposed to secondhand smoke **inside your home**?

   Never | Rarely (less than once/week) | Often (1-6 days/week) | Every day

5. Think about other places you go on a regular basis. (For example, a friend or relative's house, your workplace, bars or restaurants, in your car or someone else's car, etc.) **In the past year**, how often have you been exposed to secondhand smoke inside **other places besides your home**?

   Never | Rarely (less than once/week) | Often (1-6 days/week) | Every day

Please see back

→

6. Does **anyone else** living in your home smoke cigarettes, cigars or a pipe?

☐ Yes

☐ No

If you answered "Yes", how many other people living in your home smoke these tobacco products?

____Enter number

What is your relationship with the smoker(s)? (Check all that apply)

____Spouse or partner

____Other

Other sources of nicotine

7. Please check whether you have used any of the following in the past week:

_____ Nicotine patches

_____ Nicorette gum or similar

_____ E-cigarettes

_____ Smokeless tobacco or chewing tobacco

_____ Other source of nicotine: _____

QUESTIONNAIRE 2 (FOR FORMER SMOKERS)

Answer this questionnaire if you used to smoke at least 1 cigarette/week and quit at least 1 month ago.

Past Smoking

1. When is the last time you smoked a cigarette? (Month, Year) ____,_____

2. How many years did you smoke at least 1 cigarette/week? ____years
(If you smoked on and off, please add up the total number of years you smoked.)

3. On the average, how many cigarettes (or packs) did you used to smoke per day?

___ cigarettes/day or ____ packs/day

Secondhand Smoke

4. Think about your home **when you were growing up (less than 18 years old).** On average, how often were you exposed to secondhand smoke inside your home? (For example, how often did your parents, guardians, or siblings smoke inside the home?)
Never Rarely (less than once/week) Often (1-6 days/week) Every day

5. Think about your home **in the past year**. How often have you been exposed to secondhand smoke **inside your home**?
Never Rarely (less than once/week) Often (1-6 days/week) Every day

6. Think about other places you go on a regular basis. (For example, a friend or relative's house, your workplace, bars or restaurants, in your car or someone else's car, etc.) **In the past year**, how often have you been exposed to secondhand smoke inside **other places besides your home**?
Never Rarely (less than once/week) Often (1-6 days/week) Every day

Please see back →

7. Does **anyone** living in your home smoke cigarettes, cigars or a pipe?
☐ Yes
☐ No

If you answered “Yes”, how many people living in your home smoke these tobacco products?

____Enter number

What is your relationship with the smoker(s)? (check all that apply)

____Spouse or partner

____Other

Other sources of nicotine

8. Please check whether you have used any of the following in the past week:

_____ Nicotine patches
_____ Nicorette gum or similar
_____ E-cigarettes
_____ Smokeless tobacco or chewing tobacco
_____ Other source of nicotine: _____

QUESTIONNAIRE 3 (FOR NONSMOKERS)

Answer this if you have never smoked more than 1 cigarette/week

The goal of this questionnaire is to determine how much exposure to secondhand smoke you have had in your lifetime.

Secondhand Smoke

1. Think about your home **when you were growing up (less than 18 years old).** On average, how often were you exposed to secondhand smoke inside your home? (For example, how often did your parents, guardians, or siblings smoke inside your home?)

   Never Rarely (less than once/week) Often (1-6 days/week) Every day

2. Think about your home **in the past year**. How often have you been exposed to secondhand smoke **inside your home**?

   Never Rarely (less than once/week) Often (1-6 days/week) Every day

3. Think about other places you go on a regular basis. (For example, a friend or relative's house, your workplace, bars or restaurants, in your car or someone else's car, etc.) **In the past year**, how often have you been exposed to secondhand smoke inside **other places besides your home**?

   Never Rarely (less than once/week) Often (1-6 days/week) Every day

4. Does **anyone** living in your home smoke cigarettes, cigars or a pipe?
   ☐ Yes ☐ No
   If you answered "Yes", how many people living in your home smoke these tobacco products?
   ____Enter number
   What is your relationship with the smoker(s)? (check all that apply)
   ____Spouse or partner
   ____Other

Please see back →

Other sources of nicotine

5. Please check whether you have used any of the following in the past week:

_____ Nicotine patches
_____ Nicorette gum or similar
_____ E-cigarettes
_____ Smokeless tobacco or chewing tobacco
_____ Other source of nicotine: _____

APPENDIX B - Medical Record Data Extraction Form

DATA COLLECTION FORM Subject Identification Number:

Date of first visit (MM/YY):_____

Date of study enrollment (MM/YY):_____

Age (years) at enrollment: _____

Height: (feet, inches): _____feet_____inches

Weight: _____pounds or _____ kg

Body mass index: _____kg/m^2

Total number of previous pregnancies: _____

Regular periods (yes/no): _____

Average menstrual cycle length: _____days

Age at first period: _____years

Sexually transmitted infections: _____(yes/no)

If yes, list:_____

"Social History" Section:

Occupation: _____

Married/single: _____

Partner/no partner? _____
Length of time with current partner: _____

Routine exercise (yes/no): _____

times/week: _____

type of exercise: _____

Hours per exercise time: _____

Routine exposure to chemicals(Yes/no):_____

If yes, list here:
_____

Diet restrictions: _____

Meals/day: _____

Current tobacco use (yes/no): _____

Packs/day _____

for how many years? _____

Ever smoked 100 cigarettes? _____

Alcohol (yes/no) _____

Alcoholic drinks/week_____

Caffeine (yes/no) _____

Caffeinated drinks/day_____

Drugs (e.g. marijuana) (yes/no) _____

Ancestry or Race _____

Consistent ovulation (yes/no)_____

Clinical measurements and records from patient visits:

These will be updated as patient is followed up during the study period. A date (MM/YY) will be included for all measurements.

Blood Pressure (date of enrollment): _____ Date: (MM/YY) _____

Weight (at date of enrollment): _____ Date: (MM/YY) _____

Height: _____

BMI (at date of enrollment) _____

Anti-Mullerian hormone level (1): _____ Date: (MM/YY) _____

Anti-Mullerian hormone level (2): _____ Date: (MM/YY) _____

Antral follicle count (1): _____ Date: (MM/YY) _____

Antral follicle count (2): _____ Date: (MM/YY) _____

Infertility diagnosis/diagnoses: (More than one line is provided for multiple infertility diagnoses)

How long has the couple been trying to conceive at baseline? _____years _____months

Consistent ovulation? _____yes _____no _____unknown

Patient's Infertility Diagnoses (e.g., male factor, PCOS, fibroids, adhesions, tubal obstructions, etc.):

_____ Date of diagnosis (MM/YY) _____

_____ Date of diagnosis (MM/YY) _____

_____ Date of diagnosis (MM/YY) _____

Any diagnosis of PCOS (past or current?) YES/NO

Comments on infertility diagnosis:

Fertility treatments and interventions, and outcomes (This form may be photocopied if necessary)

IVF (1) : Date of IVF procedure (MM/YY): _____

ICSI (Intracytoplasmic sperm injection?) **YES/NO**

Donor egg used? **YES/NO**

Type of ovarian stimulation (circle): **ORAL** **INJECTABLE** **BOTH**

Oocyte yield (the number of oocytes retrieved following ovarian stimulation):

_____

Number of zygotes created _____

Number of embryos implanted _____

Outcome of procedure (clinical pregnancy: yes/no) _____

IVF (2) : Date of IVF procedure (MM/YY): _____

ICSI (Intracytoplasmic sperm injection?) **YES/NO**

Donor egg used? **YES/NO**

Type of ovarian stimulation (circle): **ORAL** **INJECTABLE** **BOTH**

Oocyte yield (the number of oocytes retrieved following ovarian stimulation):

_____

Number of zygotes created _____

Number of embryos implanted_____

Outcome of procedure (ongoing pregnancy: yes/no) _____

List other fertility interventions, date of treatment, and outcomes below:

Examples: Ovulation induction (OI) + timed intercourse, OI + IUI (intrauterine insemination), surgical interventions

1. Intervention: _____

Date (MM/YY)_____

Type of ovarian stimulation (circle): **ORAL** **INJECTABLE** **BOTH**

Outcome of procedure (ongoing pregnancy: yes/no) _____

2. Intervention: _____

Date (MM/YY)_____

Type of ovarian stimulation (circle): **ORAL** **INJECTABLE** **BOTH**

Outcome of procedure (ongoing pregnancy: yes/no) _____

Did this patient become pregnant during the follow-up period (3 years from signing consent form)?

YES/NO

If YES, list estimated month and year of

LMP (MM/YY):_____ or

Conception (MM/YY):_____ or

Due date (MM/YY):_____

Pregnancy outcome (circle on)

(1) Live birth
(2) Early pregnancy loss or Miscarriage
(3) Ectopic pregnancy
(4) Molar pregnancy
(5) Stillbirth or fetal death

Birthweight _____

IF YES, what was the treatment or intervention that resulted in ongoing pregnancy? (or did she conceive without assistance?)

_____

APPENDIX C - Recontact Questionnaire

Call script and Data Entry Form **Participant**

ID_____

Instructions: Call participants at least 24 hours after sending introductory email. Using phone numbers provided on informed consent form.

If it goes to voicemail:

Hello, my name is _____ and I am calling from the University of Louisville school of public health.

WE hope you received the email that we sent to you. In the past 18 months, you participated in a fertility study at the U of L fertility clinic. To jog your memory, it was called the LOUSSI study, and you answered a short questionnaire and provided a urine sample. I am calling today to ask if you are still trying to conceive or if you have already conceived. This will take less than 5 minutes of your time. We will try to call you again in the next week. You can also call us at 502-852-4063 and leave us a voicemail with your name, phone number and most convenient time to call. Thank you very much again for your time.

If someone answers:

Hello, my name is _____ and I am calling from the University of Louisville school of public health. May I please speak with _____?

Hello, I'm the calling from the LOUSSI study that you participated in, at the U of L fertility clinic. You answered a short smoking questionnaire and provided a urine sample. At your enrollment you gave us permission to contact you again and I am calling today to ask you a few follow-up questions. This will take less than 5 minutes of your time. Is now a good time?

If they say no- now is not a good time:

"Okay, no problem. When is a better time?" _____

Or, Would you prefer us to send you an email with the questions? (get email address).

Email address: _____

If they agree to talk:

1. Have you conceived since enrollment in the LOUSSI study? (YES or NO)
   (if No, ask questions on this page) (if Yes, ask questions on the back)
2. Are you still trying to conceive? (YES or NO)

(if NO, ask #3 and 4)

3. How many months did you continue to try after being seen at the clinic? _____months

4. Are you doing anything now to prevent pregnancy? (such as taking birth control pills?) (Y/N)

Thank you so much for your time! We really appreciate your participation in the study. Goodbye!

FOR THOSE WHO HAVE CONCEIVED:

5. How many times have you conceived since enrollment? __ times

6. (Let's talk about the *first* time you became pregnant—if they have become pregnant more than once.) How did you become pregnant?

   a. Did you conceive 'naturally'? (Yes/no)

   If not, how did you conceive?

   b. Using ovulation induction? _____

   c. Using intrauterine insemination? _____

   d. Using IVF or ICSI? _____

   If yes- Did you use donor eggs? _____

7. When did you become pregnant—in other words, what was the date of the last menstrual period before your pregnancy? _____

   (if they don't remember LMP, ask for due date.)

8. Are you still pregnant now? (Y or N)

If No: 8a. What the pregnancy outcome? (live birth, miscarriage, ectopic pregnancy, stillbirth, other)

Thank you so much for your time! We really appreciate your participation in the study. Goodbye!

APPENDIX D - Supplemental Tables

S Table 1: Characteristic of Women by Current Smoking among non-MAR Users (N=130)

	Nonsmoker N=87	Current Smoker N=43	P - value
Age in years, median (Q_1-Q_3)	32.00 (27.00-36.00)	31.00 (27.00-34.00)	0.23
BMI (kg/m^2) median (Q_1-Q_3)	30.44 (24.58-36.78)	30.64 (25.93-34.65)	0.82
BMI kg/m^2, n (%)			0.83
<25 (Underweight and Normal)	24(27.59)	10(23.26)	
25 - 29.9 (Overweight)	19(21.84)	9(20.93)	
≥ 30 (Obese)	44(50.57)	24(55.81)	
Gravidity, n (%)			0.38
0	37(42.53)	14(32.56)	
1	16(18.39)	12(27.91)	
>=2	34(39.08)	17(39.53)	
Regular Period, n (%)			0.71
No	30(34.48)	17(39.53)	
Yes	57(65.52)	26(60.47)	
Average Cycle Length (days), median (Q_1-Q_3)	28.00 (27.00-30.00)	30.00 (28.75-45.00)	<0.001
Age of Menarche, in years, median (Q_1-Q_3)	13.00 (12.00-14.00)	12.00 (11.00-13.00)	0.23
Previous STD, n (%)			0.03
No	57(65.52)	18(43.90)	
Yes	30(34.48)	23(56.10)	
Marital Status, n (%)			<0.001
No	28(32.18)	30(69.77)	
Yes	59(67.82)	13(30.23)	

Exercise, n (%)			0.18
No	36(43.90)	23(58.97)	
Yes	46(56.10)	16(41.03)	
Alcohol Use, n (%)			0.07
No	60(68.97)	22(51.16)	
Yes	27(31.03)	21(48.84)	
Caffeine Use, n (%)			0.49
No	18(21.18)	6(14.29)	
Yes	67(78.82)	36(85.71)	
Race, n (%)			0.021
Other	60(68.97)	20(46.51)	
Black	27(31.03)	23(53.49)	
AMH (ng/mL), median (Q1-Q3)	2.60 (0.92-5.09)	2.48 (1.67-6.05)	0.39
Length of Infertility, years median (Q1-Q3)	2.08 (1.50-4.63)	2.00 (1.38-5.00)	0.90
NAT2 Acetylator Status, n (%)			0.53
Rapid	44(58.67)	26(66.67)	
Slow	31(41.33)	13(33.33)	
Pregnant, n (%)			0.97
No	67(77.01)	34(79.07)	
Yes	20(22.99)	9(20.93)	
Pregnancy Outcome, n (%)			0.021
Live Birth	13(81.25)	2(25.00)	
Miscarriage	3(18.75)	6(75.00)	
Total Follow-up Time in years, median (Q1-Q3)	8.00(4.00-15.00)	8.00(2.00-15.50)	0.58
Cotinine Levels mg/dL, median (Q1-Q3)	0.50(0.00-4.38)	100(100-100)	< 0.001
Cotinine Categories, n (%)			< 0.001
0 mg/dL	38(46.34)	0(0.00)	
<100 mg/dL	44(53.66)	2(4.76)	
100mg/dL	0(0.00)	40(95.24)	

S Table 2: Characteristic of Women by Current Smoking among MAR Users (N=93)

	Nonsmoker N=81	Current Smoker N=11	P - value
Age (years) median (Q_1-Q_3)	33.00 (29.25-37.00)	36.00 (34.50-38.50)	0.06
BMI, (mean ± SD), (kg/m^2)	29.01 ± 6.14	30.83 ± 4.98	0.35
BMI kg/m^2, n (%)			0.43
<25 (Underweight and Normal)	22(26.83)	1(9.09)	
25 - 29.9 (Overweight)	30(36.59)	4(36.36)	
≥ 30 (Obese)	30(36.59)	6(54.55)	
Gravidity, n (%)			0.07
0	37(45.12)	5(45.45)	
1	22(26.83)	0(0.00)	
>=2	23(28.05)	6(54.55)	
Regular Period, n (%)			0.16
No	28(34.15)	1(9.09)	
Yes	54(65.85)	10(90.91)	
Average Cycle Length (days), median (Q_1-Q_3)	29.00 (28.00-30.00)	28.50 (28.00-29.00)	0.62
Age of Menarche, in years, median (Q_1-Q_3)	13.00 (12.00-14.00)	13.00 (11.50-13.00)	0.37
Previous STD, n (%)			1
No	50(61.73)	7(63.64)	
Yes	31(38.27)	4(36.36)	
Marital Status, n (%)			0.01
No	18(21.95)	7(63.64)	
Yes	64(78.05)	4(36.36)	
Exercise, n (%)			0.75

No	31(38.75)	5(45.45)	
Yes	49(61.25)	6(54.55)	
Alcohol Use, n (%)			0.34
No	37(45.12)	7(63.64)	
Yes	45(54.88)	4(36.36)	
Caffeine Use, n (%)			1
No	16(19.51)	2(18.18)	
Yes	66(80.49)	9(81.82)	
Race, n (%)			0.21
Other	68(82.93)	7(63.64)	
Black	14(17.07)	4(36.36)	
AMH (ng/mL), median (Q1-Q3)	2.44 (1.38-4.99)	2.30 (1.35-3.16)	0.66
Length of Infertility, years median (Q1-Q3)	1.46 (0.94-4.33)	2.50 (1.58-7.00)	0.06
NAT2 Acetylator Status, n (%)			0.51
Rapid	40(57.14)	7(70.00)	
Slow	30(42.86)	3(30.00)	
Pregnant, n (%)			1
No	34(41.46)	5(45.45)	
Yes	48(58.54)	6(54.55)	
Pregnancy Outcome, n (%)			0.39
Live Birth	24(57.14)	2(33.33)	
Miscarriage	18(42.86)	4(66.67)	
Total Follow-up Time, years, median (Q1-Q3)	5.00 (3.00-12.00)	7.00 (3.50-8.00)	0.85
Cotinine Levels, mg/dL median (Q1-Q3)	0.00 (0.00-2.00)	100 (100-100)	<0.001
Cotinine mg/dL, n (%)			<0.001
0	44(56.41)	0(0.00)	
<100	34(43.59)	0(0.00)	
100	0(0.00)	10(100.00)	

S Table 3: Characteristics of Nonsmoking Women Stratified by Recent SHS Exposure Measured With Urinary Cotinine among MAR Users (N=78)

	Cotinine = 0 mg/dL N=44	0 < Cotinine ≤ 4mg/dL N=19	4 < Cotinine ≤ 47 mg/dL N=15	P-value
Age, years (mean ± SD)	33.66 ± 5.26	32.89 ± 4.32	31.67 ± 5.30	0.42
BMI, kg/m2 (mean ± SD)	27.77 ± 6.19	30.18 ± 4.39	32.06 ± 7.25	0.05
BMI kg/m2, n (%)				0.44
<25 (Underweight and Normal)	15(34.09)	3(15.79)	3(20.00)	
25 - 29.9 (Overweight)	15(34.09)	8(42.11)	4(26.67)	
≥ 30 (Obese)	14(31.82)	8(42.11)	8(53.33)	
Gravidity, n (%)				0.011
0	17(38.64)	14(73.68)	3(20.00)	
1	11(25.00)	2(10.53)	8(53.33)	
>=2	16(36.36)	3(15.79)	4(26.67)	
Regular Period, n (%)				0.40
No	12(27.27)	8(42.11)	6(40.00)	
Yes	32(72.73)	11(57.89)	9(60.00)	
Average Cycle Length (days), median (Q_1-Q_3)	28.50 (28.00-30.00)	29.00 (28.00-29.88)	28.50 (26.50-29.75)	0.84
Age of Menarche, in years, median (Q_1-Q_3)	13.00 (12.00-14.00)	12.50 (11.25-13.75)	12.00 (12.00-13.50)	0.89
Previous STD, n (%)				0.73
No	27(61.36)	12(66.67)	8(53.33)	
Yes	17(38.64)	6(33.33)	7(46.67)	

Marital Status, n (%)				0.32
No	7(15.91)	5(26.32)	5(33.33)	
Yes	37(84.09)	14(73.68)	10(66.67)	
Exercise, n (%)				0.57
No	14(33.33)	9(47.37)	6(40.00)	
Yes	28(66.67)	10(52.63)	9(60.00)	
Alcohol Use, n (%)				0.45
No	17(38.64)	10(52.63)	8(53.33)	
Yes	27(61.36)	9(47.37)	7(46.67)	
Caffeine Use, n (%)				1
No	9(20.45)	3(15.79)	3(20.00)	
Yes	35(79.55)	16(84.21)	12(80.00)	
Race, n (%)				0.81
White	32(72.73)	12(63.16)	10(66.67)	
Black	6(13.64)	5(26.32)	3(20.00)	
Other	6(13.64)	2(10.53)	2(13.33)	
AMH (ng/mL), median (Q1-Q3)	2.31 (1.10-4.50)	3.23 (1.34-5.26)	2.52 (1.87-4.44)	0.73
Length of Infertility, years median (Q1-Q3)	1.04 (0.88-3.33)	1.50 (1.19-5.50)	2.21 (0.81-5.06)	0.72
Treatment Cycles, n (%)				0.04
1	17(38.64)	7(36.84)	10(66.67)	
2	12(27.27)	4(21.05)	5(33.33)	
3 and more	15(34.09)	8(42.11)	0(0.00)	
NAT2 Acetylator Status, n (%)				0.40
Rapid	25(64.10)	8(47.06)	7(50.00)	
Slow	14(35.90)	9(52.94)	7(50.00)	
Pregnant, n (%)				0.50
No	20(45.45)	6(31.58)	5(33.33)	
Yes	24(54.55)	13(68.42)	10(66.67)	
Pregnancy Outcome, n (%)				0.036

Live Birth	16(72.73)	5(50.00)	2(22.22)	
Miscarriage	6(27.27)	5(50.00)	7(77.78)	
Total Follow-up Time, years median (Q1-Q3)	6.00 (3.00-15.00)	6.00 (4.00-13.00)	3.00 (2.00-10.50)	0.17

S Table 4: Characteristics of Nonsmoking Women Stratified by Recent SHS Exposure Measured with Urinary Cotinine among non-MAR Users (N=82)

	Cotinine = 0 mg/dL N=38	**0 < Cotinine ≤ 4mg/dL N=23**	**4 < Cotinine ≤ 47 mg/dL N=21**	**P-value**
Age, years (mean ± SD)	33.47 ± 5.12	30.87 ± 6.06	31.00 ± 5.82	0.13
BMI, kg/m2 (mean ± SD)	27.49 (23.89-32.44)	35.51 (27.61-41.33)	30.64 (24.19-36.11)	0.02
BMI kg/m2, n (%)				0.24
<25 (Underweight and Normal)	11(28.95)	4(17.39)	6(28.57)	
25 - 29.9 (Overweight)	12(31.58)	3(13.04)	4(19.05)	
≥ 30 (Obese)	15(39.47)	16(69.57)	11(52.38)	
Gravidity, n (%)				0.02
0	13(34.21)	15(65.22)	7(33.33)	
1	8(21.05)	5(21.74)	2(9.52)	
>=2	17(44.74)	3(13.04)	12(57.14)	
Regular Period, n (%)				0.41
No	13(34.21)	11(47.83)	6(28.57)	
Yes	25(65.79)	12(52.17)	15(71.43)	
Average Cycle Length (days), median (Q_1-Q_3)	28.00 (27.0-30.0)	28.00 (27.0-28.0)	29.00 (27.5-30.0)	0.52
Age of Menarche, in years, median (Q_1-Q_3)	13(12-14)	12(11-13.5)	13(12-14)	0.43
Previous STD, n (%)				1

No	25(65.79)	15(65.22)	14(66.67)	
Yes	13(34.21)	8(34.78)	7(33.33)	
Marital Status, n (%)				0.15
No	14(36.84)	4(17.39)	9(42.86)	
Yes	24(63.16)	19(82.61)	12(57.14)	
Routine Exercise, n (%)				0.04
No	13(35.14)	7(33.33)	13(68.42)	
Yes	24(64.86)	14(66.67)	6(31.58)	
Alcohol Use, n (%)				0.64
No	28(73.68)	16(69.57)	13(61.90)	
Yes	10(26.32)	7(30.43)	8(38.10)	
Caffeine Use, n (%)				0.56
No	10(26.32)	3(13.04)	4(21.05)	
Yes	28(73.68)	20(86.96)	15(78.95)	
Race, n (%)				0.89
White	15(39.47)	9(39.13)	8(38.10)	
Black	10(26.32)	7(30.43)	8(38.10)	
Other	13(34.21)	7(30.43)	5(23.81)	
AMH (ng/mL), median (Q1-Q3)	1.98 (3.78-9.71)	3.28 (0.93-5.85)	2.87 (1.04-6.93)	0.41
Length of Infertility, years median (Q1-Q3)	2.00 (1.50-4.00)	2.08 (1.50-3.38)	2.50 (1.19-5.75)	0.96
NAT2 Acetylator Status, n (%)				0.04
Rapid	22(64.71)	15(71.43)	7(35.00)	
Slow	12(35.29)	6(28.57)	13(65.00)	
Pregnant, n (%)				0.89
No	29(76.32)	17(73.91)	17(80.95)	
Yes	9(23.68)	6(26.09)	4(19.05)	
Pregnancy Outcome, n (%)				1
Live Birth	5(71.43)	4(80.00)	3(100.00)	
Miscarriage	2(28.57)	1(20.00)	0(0.00)	
Total Follow-up Time, years median (Q1-Q3)	11.00 (7.00-16.00)	4.00 (3.00-14.5)	5.00 (3.00-9.00)	0.04

S Table 5: Characteristics of Nonsmoking Women Stratified by SHS Exposure Measured from SSQ among MAR Users (N=82)

	No and Low exposure (score=2,3) N=62	Med and High exposure (score ≥4) N=20	P-value
Age, years (mean ± SD)	32.44 ± 5.15	34.2 ± 5.04	0.18
BMI, kg/m2 (mean ± SD)	28.22 ± 5.58	31.47 ± 7.22	0.04
BMI Category kg/m2, n (%)			0.65
<25 (Underweight and Normal)	17(27.42)	5(25.00)	
25 - 29.9 (Overweight)	24(38.71)	6(30.00)	
≥ 30 (Obese)	21(33.87)	9(45.00)	
Gravidity, n (%)			0.85
0	29(46.77)	8(40.00)	
1	16(25.81)	6(30.00)	
>=2	17(27.42)	6(30.00)	
Regular Period, n (%)			0.36
No	19(30.65)	9(45.00)	
Yes	43(69.35)	11(55.00)	
Average Cycle Length (days), median (Q_1-Q_3)	29.00 (28.00-30.00)	28.00 (26.25-30.00)	0.43
Age of Menarche, in years, median (Q_1-Q_3)	13.00 (12.00-14.00)	12.00 (12.00-13.00)	0.14
Previous STD, n (%)			1
No	38(62.30)	12(60.00)	
Yes	23(37.70)	8(40.00)	
Marital Status, n (%)			0.03
No	10(16.13)	8(40.00)	
Yes	52(83.87)	12(60.00)	
Exercise, n (%)			1
No	23(38.33)	8(40.00)	

Yes	37(61.67)	12(60.00)	
Alcohol Use, n (%)			0.45
No	26(41.94)	11(55.00)	
Yes	36(58.06)	9(45.00)	
Caffeine Use, n (%)			1
No	12(19.35)	4(20.00)	
Yes	50(80.65)	16(80.00)	
Race, n (%)			0.63
White	44(70.97)	12(60.00)	
Black	10(16.13)	4(20.00)	
Other	8(12.90)	4(20.00)	
AMH (ng/mL), median (Q1-Q3)	2.44 (1.34-4.98)	2.40 (1.55-5.47)	0.86
Length of Infertility, years median (Q1-Q3)	1.02 (0.31-2.77)	2.52 (0.77-7.00)	0.04
Treatment Cycles, n (%)			0.27
1	25(40.32)	10(50.00)	
2	16(25.81)	7(35.00)	
3 and more	21(33.87)	3(15.00)	
NAT2 Acetylator Status, n (%)			0.27
Rapid	32(61.54)	8(44.44)	
Slow	20(38.46)	10(55.56)	
Pregnant, n (%)			0.53
No	24(38.71)	10(50.00)	
Yes	38(62.30)	10(50.00)	
Pregnancy Outcome, n (%)			0.01
Live Birth	22(68.75)	2(20.00)	
Miscarriage	10(31.25)	8(80.00)	
Total Follow-up Time, years median (Q1-Q3)	5.00(3.00-12.00)	5.50(3.75-12.25)	0.55
Cotinine Levels, mg/dL median (Q1-Q3)	0.00(0.00-1.00)	1.25(0.00-15.63)	0.03
Cotinine mg/dL, n (%)			0.01
0	35(60.34)	9(45.00)	
<4	16(27.59)	2(10.00)	

	>= 4	7(12.07)	9(45.00)

S Table 6: Characteristics of Nonsmoking Women Stratified by SHS Exposure Measured from SSQ among non-MAR Users (N=87)

	No and Low exposure (score=2,3) N=50	Med and High exposure (score ≥4) N=37	P-value
Age, years (mean ± SD)	33.16 ± 5.72	30.73 ± 5.23	0.045
BMI, kg/m2 median (Q_1-Q_3)	30.45 (24.75-35.02)	29.04 (24.14-37.25)	1
BMI kg/m2, n (%)			0.93
<25 (Underweight and Normal)	13(26.00)	11(29.73)	
25 - 29.9 (Overweight)	11(22.00)	8(21.62)	
≥ 30 (Obese)	26(52.00)	18(48.65)	
Gravidity, n (%)			0.61
0	19(38.00)	18(48.65)	
1	10(20.00)	6(16.22)	
>=2	21(42.00)	13(35.14)	
Regular Period, n (%)			1
No	17(34.00)	13(35.14)	
Yes	33(66.00)	24(64.86)	
Average Cycle Length (days), median (Q_1-Q_3)	28.00 (26.00-29.25)	28.00 (28.00-30.00)	0.34
Age of Menarche, in years, median (Q_1-Q_3)	12.00 (11.75-14.00)	13.00 (12.00-14.00)	0.95
Previous STD, n (%)			0.74
No	34(68.00)	23(62.16)	
Yes	16(32.00)	14(37.84)	
Marital Status, n (%)			0.23
No	13(26.00)	15(40.54)	
Yes	37(74.00)	22(59.46)	

Exercise, n (%)			0.16
No	17(36.17)	19(54.29)	
Yes	30(63.83)	16(45.71)	
Alcohol Use, n (%)			1
No	34(68.00)	26(70.27)	
Yes	16(32.00)	11(29.73)	
Caffeine Use, n (%)			1
No	10(20.41)	8(22.22)	
Yes	39(79.59)	28(77.78)	
Race, n (%)			0.02
White	19(38.00)	16(43.24)	
Black	11(22.00)	16(43.24)	
Other	20(40.00)	5(13.51)	
AMH (ng/mL), median (Q1-Q3)	2.48 (0.81-3.84)	2.67 (0.99-6.28)	0.52
Length of Infertility, years median (Q1-Q3)	2.00 (1.04-4.00)	3.00 (1.03-5.00)	0.73
NAT2 Acetylator Status, n (%)			0.79
Rapid	23(56.10)	21(61.76)	
Slow	18(43.90)	13(38.24)	
Pregnant, n (%)			0.99
No	38(76.00)	29(78.38)	
Yes	12(24.00)	8(21.62)	
Pregnancy Outcome, n (%)			1
Live Birth	7(77.78)	6(85.71)	
Miscarriage	2(22.22)	1(14.29)	
Total Follow-up Time, years median (Q1-Q3)	9.00 (4.25-10.48)	5.00 (3.00-13.00)	0.13
Cotinine Levels, mg/dL median (Q1-Q3)	0.00 (0.00-1.00)	4.00 (0.50-9.10)	<0.001
Cotinine mg/dL, n (%)			<0.001
0	30(63.83)	8(22.86)	
<4	10(21.28)	9(25.71)	
>= 4	7(14.89)	18(51.43)	

S Table 7: Characteristics of Women that used ART Measured Using the 3-level Combined Effect Variable among MAR users (N=93)

	Nonsmokers with Low SHS N=62	Nonsmokers with High SHS N=20	Smokers N=11	P - value
Age, years median (Q_1-Q_3)	33.00 (29.00-37.00)	35.00 (31.00-38.25)	36.00 (34.50-38.50)	0.07
BMI, kg/m2 (mean ± SD)	28.22 ± 5.58	31.47 ± 7.22	30.83 ± 4.98	0.07
BMI Category, kg/m2, n (%)				0.61
<25 (Underweight and Normal)	17(27.42)	5(25.00)	1(9.09)	
25 - 29.9 (Overweight)	24(38.71)	6(30.00)	4(36.36)	
≥ 30 (Obese)	21(33.87)	9(45.00)	6(54.55)	
Gravidity, n (%)				0.22
0	29(46.77)	8(40.00)	5(45.45)	
1	16(25.81)	6(30.00)	0(0.00)	
>=2	17(27.42)	6(30.00)	6(54.55)	
Regular Period, n (%)				0.12
No	19(30.65)	9(45.00)	1(9.09)	
Yes	43(69.35)	11(55.00)	10(90.91)	
Average Cycle Length (days), median (Q_1-Q_3)	29.00 (28.00-30.00)	28.00 (26.25-30.00)	28.50 (28.00-29.00)	0.63
Age of Menarche, years, median (Q_1-Q_3)	13.00 (12.00-14.00)	12.00 (12.00-13.00)	13.00 (11.50-13.00)	0.19
Previous STD, n (%)				1
No	38(62.30)	12(60.00)	7(63.64)	
Yes	23(37.70)	8(40.00)	4(36.36)	

Marital Status, n (%)				0.002
No	10(16.13)	8(40.00)	7(63.64)	
Yes	52(83.87)	12(60.00)	4(36.36)	
Exercise, n (%)				0.91
No	23(38.33)	8(40.00)	5(45.45)	
Yes	37(61.67)	12(60.00)	6(54.55)	
Alcohol Use, n (%)				0.31
No	26(41.94)	11(55.00)	7(63.64)	
Yes	36(58.06)	9(45.00)	4(36.36)	
Caffeine Use, n (%)				1
No	12(19.35)	4(20.00)	2(18.18)	
Yes	50(80.65)	16(80.00)	9(81.82)	
Race, n (%)				0.29
Black	10(16.13)	4(20.00)	4(36.36)	
Other	52(83.87)	16(80.00)	7(63.64)	
AMH (ng/mL), median (Q1-Q3)	2.44 (1.34-4.98)	2.40 (1.55-5.47)	2.30 (1.35-3.16)	0.89
Length of Infertility, years median (Q1-Q3)	1.21 (0.94-2.94)	2.75 (0.94-7.00)	2.50 (1.58-7.00)	0.02
Treatment Cycles, n (%)				0.39
1	25(40.32)	10(50.00)	3(27.27)	
2	16(25.81)	7(35.00)	3(27.27)	
3 and more	21(33.87)	3(15.00)	5(45.45)	
NAT2 Acetylator Status, n (%)				0.33
Rapid	32(61.54)	8(44.44)	7(70.00)	
Slow	20(38.46)	10(55.56)	3(30.00)	
Pregnant, n (%)				0.66
No	24(38.71)	10(50.00)	5(45.45)	
Yes	38(61.29)	10(50.00)	6(54.55)	
Pregnancy Outcome, n (%)				0.01
Live Birth	22(68.75)	2(20.00)	2(33.33)	
Miscarriage	10(31.25)	8(80.00)	4(66.67)	

Total Follow-up Time, years median (Q1-Q3)	5.00 (12.00-3.00)	5.50 (12.25-3.75)	7.00 (8.00-3.50)	0.81
Cotinine Levels, mg/dL median (Q1-Q3)	0.00 (0.00-1.00)	1.25 (0.00-15.63)	100.00 (100.0-100.0)	<0.001
Cotinine mg/dL, n (%)				<0.001
0	35(60.34)	9(45.00)	0(0.00)	
<4	23(39.66)	11(55.00)	0(0.00)	
>= 4	0(0.00)	0(0.00)	10(100.00)	

S Table 8: Characteristics of Women that used ART Measured Using the 3-level Combined Effect Variable among non-MAR Users (N=130)

	Nonsmokers with low SHS N=50	**Nonsmokers with high SHS N=37**	**Smokers N=43**	**P - value**
Age, years median (Q_1-Q_3)	34.00 (29.00-37.75)	31.00 (26.00-35.00)	31.00 (27.00-34.00-)	0.07
BMI, kg/m^2 median (Q_1-Q_3)	30.45 (24.75-35.02)	29.04 (24.14-37.25)	30.64 (25.93-34.65)	0.97
BMI, kg/m^2, n (%)				0.97
<25 (Underweight and Normal)	13(26.00)	11(29.73)	10(23.26)	
25 - 29.9 (Overweight)	11(22.00)	8(21.62)	9(20.93)	
≥ 30 (Obese)	26(52.00)	18(48.65)	24(55.81)	
Gravidity, n (%)				0.59
0	19(38.00)	18(48.65)	14(32.56)	
1	10(20.00)	6(16.22)	12(27.91)	
>=2	21(42.00)	13(35.14)	17(39.53)	
Regular Period, n (%)				0.85
No	17(34.00)	13(35.14)	17(39.53)	

Yes	33(66.00)	24(64.86)	26(60.47)	
Average Cycle Length (days), median (Q_1-Q_3)	28.00 (26.00-29.25)	28.00 (28.00-30.00)	30.00 (28.75-31.25)	0.001
Age of Menarche, years, median (Q_1-Q_3)	12.00 (11.75-14.00)	13.00 (12.00-14.00)	12.00 (11.00-13.00)	0.47
Previous STD, n (%)				0.06
No	34(68.00)	23(62.16)	18(43.90)	
Yes	16(32.00)	14(37.84)	23(56.10)	
Marital Status, n (%)				<0.001
No	13(26.00)	15(40.54)	30(69.77)	
Yes	37(74.00)	22(59.46)	13(30.23)	
Exercise, n (%)				0.08
No	17(36.17)	19(54.29)	23(58.97)	
Yes	30(63.83)	16(45.71)	16(41.03)	
Alcohol Use, n (%)				0.14
No	34(68.00)	26(70.27)	22(51.16)	
Yes	16(32.00)	11(29.73)	21(48.84)	
Caffeine Use, n (%)				0.63
No	10(20.41)	8(22.22)	6(14.29)	
Yes	39(79.59)	28(77.78)	36(85.71)	
Race, n (%)				0.006
Black	11(22.00)	16(43.24)	23(53.49)	
Other	39(78.00)	21(56.76)	20(46.51)	
AMH (ng/mL), median (Q1-Q3)	2.48 (0.81-3.84)	2.67 (0.99-6.28)	2.48 (1.67-6.05)	0.57
Length of Infertility, years median (Q1-Q3)	2.00 (1.50-4.00)	3.00 (1.33-5.00)	2.00 (1.38-5.00)	0.93
NAT2 Acetylator Status, n (%)				0.62
Rapid	23(56.10)	21(61.76)	26(66.67)	
Slow	18(43.90)	13(38.24)	13(33.33)	
Pregnant, n (%)				0.93

No	38(76.00)	29(78.38)	34(79.07)	
Yes	12(24.00)	8(21.62)	9(20.93)	
Pregnancy Outcome, n (%)				0.04
Live Birth	7(77.78)	6(85.71)	2(25.00)	
Miscarriage	2(22.22)	1(14.29)	6(75.00)	
Total Follow-up Time, years median (Q1-Q3)	9.00 (4.25-16.00)	5.00 (3.00-13.00)	8.00 (2.00-15.50)	0.29
Cotinine Levels, mg/dL median (Q1-Q3)	0.00 (0.00-1.00)	4.00 (0.50-9.10)	100.00 (100.0-100.0)	< 0.001
Cotinine mg/dL, n (%)				< 0.001
0	30(63.83)	8(22.86)	0(0.00)	
<4	17(36.17)	27(77.14)	2(4.76)	
>= 4	0(0.00)	0(0.00)	40(95.24)	

S Table 9: Characteristics of Women from Group 1 Stratified by Type of Follow-up (None vs Medical Records) (N=95)

	No Follow Up N=21	**Medical Record (MR) N=74**	**P-value**
Age, years, (mean ± SD)	30.95 ± 7.28	32.35 ± 5.17	0.42
BMI, kg/m$_2$, median (Q_1-Q_3)	32.20 (29.35-42.89)	30.32 (26.59-35.97)	0.11
BMI kg/m$_2$, n (%)			0.89
<25 (Underweight and Normal)	3(15.00)	14(18.92)	
25 - 29.9 (Overweight)	5(25.00)	21(28.38)	
≥ 30 (Obese)	12(60.00)	39(52.70)	
Gravidity, n (%)			0.71

0	6(28.57)	29(39.19)	
1	7(33.33)	21(28.38)	
>=2	8(38.10)	24(32.43)	
Regular Period, n (%)			0.24
No	11(52.38)	26(35.14)	
Yes	10(47.62)	48(64.86)	
Average Cycle Length, days, median (Q_1-Q_3)	28 (26.50-29.75)	29.00 (28.00-30.00)	0.29
Age of Menarche, (years) median(Q_1-Q_3)	12(11-13)	12(12-14)	0.11
Previous STD, n (%)			0.70
No	14(70.00)	46(62.16)	
Yes	6(30.00)	28(37.84)	
Marital Status, n (%)			0.31
No	9(45.00)	22(29.73)	
Yes	11(55.00)	52(70.27)	
Routine Exercise, n (%)			0.40
No	11(57.89)	31(43.66)	
Yes	8(42.11)	40(56.34)	
Alcohol Use, n (%)			0.74
No	11(52.38)	44(59.46)	
Yes	10(47.62)	30(40.54)	
Caffeine Use, n (%)			0.39
No	3(15.00)	19(25.68)	
Yes	17(85.00)	55(74.32)	
Race, n (%)			0.24
Black	10(47.62)	48(64.86)	
Other	11(52.38)	26(35.14)	
AMH (ng/mL), median (Q1-Q3)	1.85 (0.67-3.79)	2.77 (1.13-5.45)	0.33
Length of Infertility, years, median(Q_1-Q_3)	3.50 (1.50-4.25)	2.00 (1.19-5.00)	0.42
NAT2 Acetylator Status, n (%)			0.91
Rapid	9(47.37)	34(52.31)	
Slow	10(52.63)	31(47.69)	

Pregnant, n (%)			<0.001
No	21(100.00)	47(63.51)	
Yes	0(0.00)	27(36.49)	
Pregnancy Outcome, n (%)			
Miscarriage	0(0.00)	12(57.14)	
Live Birth	0(0.00)	9(42.86)	
Cotinine mg/dL, median (Q_1-Q_3)	28.00 (2.00-100)	4.00 (1.00-19.75)	0.13
Cotinine mg/dL, n (%)			0.04
0	4(19.05)	13(18.06)	
<100	7(33.33)	44(61.11)	
100	10(47.62)	15(20.83)	
Current, Active Smoker, n (%)			0.04
No	11(52.38)	58(78.38)	
Yes	10(47.62)	16(21.62)	
Self-reported SHS Exposure, n (%)			0.35
Low	8(38.10)	39(52.70)	
High	13(61.90)	35(47.30)	

S Table 10: Characteristics of Women from Group 2 Stratified by Type of Follow-up (None vs Medical Records vs Personal) (N=162)

	No Follow Up N=7	**Medical Record (MR) only N=35**	**Personal only N=120**	**P-value**
Age, years, median (Q_1-Q_3)	29.50 (26.00-37.50)	33.00 (26.00-34.00)	33.00 (28.00-37.00)	0.45
BMI, kg/m$_2$, median (Q_1-Q_3)	22.80 (20.39-32.73)	28.72 (25.16-34.30)	28.54 (23.62-34.39)	0.58
BMI kg/m$_2$, n (%)				0.25

<25 (Underweight and Normal)	4(66.67)	8(22.86)	39(32.50)	
25 - 29.9 (Overweight)	0(0.00)	12(34.29)	30(25.00)	
≥ 30 (Obese)	2(33.33)	15(42.86)	51(42.50)	
Gravidity, n (%)				0.57
0	1(16.67)	17(48.57)	51(42.50)	
1	1(16.67)	7(20.00)	23(19.17)	
>=2	4(66.67)	11(31.43)	46(38.33)	
Regular Period, n (%)				0.36
No	3(50.00)	9(25.71)	43(35.83)	
Yes	3(50.00)	26(74.29)	77(64.17)	
Average Cycle Length, days, median (Q_1-Q_3)	28.0 (28.00-29.00)	28.5 (28.00-30.00)	28.7 (27.00-30.00)	0.78
Age of Menarche, (years) median(Q_1-Q_3)	12 (11.25-12.75)	13 (11.00-13.00)	12.5 (11.00-14.00)	0.75
Previous STD, n (%)				0.04
No	3(50.00)	27(77.14)	63(54.31)	
Yes	3(50.00)	8(22.86)	53(45.69)	
Marital Status, n (%)				0.74
No	2(33.33)	16(45.71)	47(39.17)	
Yes	4(66.67)	19(54.29)	73(60.83)	
Routine Exercise, n (%)				0.05
No	2(33.33)	16(45.71)	47(39.17)	
Yes	4(66.67)	19(54.29)	73(60.83)	
Alcohol Use, n (%)				0.03
No	5(83.33)	25(71.43)	60(50.00)	
Yes	1(16.67)	10(28.57)	60(50.00)	
Caffeine Use, n (%)				0.7
No	0(0.00)	6(18.18)	20(16.81)	
Yes	6(100.00)	27(81.82)	99(83.19)	
Race, n (%)				0.10
Black	2(33.33)	27(77.14)	83(69.17)	
Other	4(66.67)	8(22.86)	37(30.83)	
AMH (ng/mL), median (Q1-Q3)	3.85 (3.06-4.65)	2.17 (1.47-3.99)	2.49 (1.27-4.99)	0.7

Length of Infertility, years, median(Q_1-Q_3)	1.00 (0.83-1.17)	2.00 (1.00-5.00)	2.00 (1.00-4.88)	0.10
NAT2 Acetylator Status, n (%)				0.1
Rapid	7(100.00)	16(45.71)	76(63.33)	
Slow	0(0.00)	8(22.86)	17(14.17)	
Pregnant, n (%)				0.21
No	7(100.00)	21(60.00)	77(64.17)	
Yes	0(0.00)	14(40.00)	43(35.83)	
Pregnancy Outcome, n (%)				
Miscarriage	0(0.00)	6(45.15)	23(58.97)	0.12
Live Birth	0(0.00)	7(53.85)	16(41.03)	
Cotinine mg/dL, median (Q_1-Q_3)	100 (27.3-100)	16.0 (0.00-100)	0.00 (0.00-32.3)	0.52
Cotinine mg/dL, n (%)				
0	1(16.67)	11(35.48)	60(52.63)	
<100	1(16.67)	10(32.26)	27(23.68)	<0.001
100	4(66.67)	10(32.26)	27(23.68)	0.03
Current, Active Smoker, n (%)				0.17
No	3(42.86)	25(71.43)	90(75.00)	
Yes	4(57.14)	10(28.57)	30(25.00)	
Self-reported SHS Exposure, n (%)				0.10
Low	1(14.29)	15(42.86)	63(52.50)	
High	6(85.71)	20(57.14)	57(47.50)	

S Table 11: Characteristics of Women from Group 2 Stratified by Type of Follow-up (Medical Records vs Personal) (N=95)

	Medical Record only N=35	Personal only N=120	P-value
Age, years, median (Q_1-Q_3)	33.00 (26.00-34.00)	33.00 (28.00-37.00)	0.23
BMI, kg/m2, median (Q_1-Q_3)	28.72 (25.16-34.30)	28.54 (23.62-34.39)	0.71
BMI kg/m2, n (%)			0.43
<25 (Underweight and Normal)	8(22.86)	39(32.50)	
25 - 29.9 (Overweight)	12(34.29)	30(25.00)	
≥ 30 (Obese)	15(42.86)	51(42.50)	
Gravidity, n (%)			0.75
0	17(48.57)	51(42.50)	
1	7(20.00)	23(19.17)	
>=2	11(31.43)	46(38.33)	
Regular Period, n (%)			0.36
No	9(25.71)	43(35.83)	
Yes	26(74.29)	77(64.17)	
Average Cycle Length, days, median (Q_1-Q_3)	28.50 (28.00-30.00)	28.50 (27.00-30.00)	0.5
Age of Menarche, (years) median(Q_1-Q_3)	13.00 (11.00-13.00)	12.50 (11.00-14.00)	0.6
Previous STD, n (%)			0.03
No	27(77.14)	63(54.31)	
Yes	8(22.86)	53(45.69)	
Marital Status, n (%)			0.62
No	16(45.71)	47(39.17)	
Yes	19(54.29)	73(60.83)	
Routine Exercise, n (%)			0.03
No	21(61.76)	44(39.29)	
Yes	13(38.24)	68(60.71)	
Alcohol Use, n (%)			0.04
No	25(71.43)	60(50.00)	

Yes	10(28.57)	60(50.00)	
Caffeine Use, n (%)			0.8
No	6(18.18)	20(16.81)	
Yes	27(81.82)	99(83.19)	
Race, n (%)			0.48
Black	27(77.14)	83(69.17)	
Other	8(22.86)	37(30.83)	
AMH (ng/mL), median (Q1-Q3)	2.17 (1.47-3.99)	2.49 (1.27-4.99)	0.52
Length of Infertility, years, median(Q_1-Q_3)	2.00 (1.00-4.67)	1.5 (0.56-4.00)	0.3
Treatment Cycles, n (%)			0.17
0	16(45.71)	76(63.33)	
1	8(22.86)	17(14.17)	
2 and more	11(31.43)	27(22.50)	
NAT2 Acetylator Status, n (%)			0.94
Rapid	20(66.67)	65(63.73)	
Slow	10(33.33)	37(36.27)	
Pregnant, n (%)			0.8
No	21(60.00)	77(64.17)	
Yes	14(40.00)	43(35.83)	
Pregnancy Outcome, n (%)			0.63
Miscarriage	6(46.15)	23(58.97)	
Live Birth	7(53.85)	16(41.03)	
Cotinine mg/dL, median (Q_1-Q_3)	16.00 (0.00-100)	0.00 (0.00-100)	0.08
Cotinine mg/dL, n (%)			0.23
0	1(16.67)	71(48.97)	
<100	1(16.67)	37(25.52)	
100	4(66.67)	37(25.52)	
Current, Active Smoker, n (%)			0.84
No	25(71.43)	90(75.00)	
Yes	10(28.57)	30(25.00)	

Self-reported SHS Exposure, n (%)				0.42
	Low	15(42.86)	63(54.31)	
	High	20(57.14)	57(47.50)	

S Table 12: Characteristics of Women from Group 2 Stratified by Type of Follow-up (None vs Any) (N=95)

	No Follow-Up N=7	MR and Personal Follow-up N=155	P-value
Age, years, median (Q_1-Q_3)	29.50 (26.00-37.50)	33.00 (27.00-37.00)	0.69
BMI, kg/m2, median (Q_1-Q_3)	22.8 (20.39-32.73)	28.54 (23.80-34.42)	0.34
BMI kg/m2, n (%)			0.15
<25 (Underweight and Normal)	4(66.67)	47(30.32)	
25 - 29.9 (Overweight)	0(0.00)	42(27.10)	
≥ 30 (Obese)	2(33.33)	66(42.58)	
Gravidity, n (%)			0.25
0	1(16.67)	68(43.87)	
1	1(16.67)	30(19.35)	
>=2	4(66.67)	57(36.77)	
Regular Period, n (%)			0.42
No	3(50.00)	52(33.55)	
Yes	3(50.00)	103(66.45)	
Average Cycle Length, days, median (Q_1-Q_3)	28.00 (28.00-29.00)	28.50 (28.00-30.00)	0.91
Age of Menarche, (years) median(Q_1-Q_3)	12.00 (11.25-12.75)	13.00 (11.0-14.0)	0.57
Previous STD, n (%)			0.69
No	3(50.00)	90(59.6)	
Yes	3(50.00)	61(40.4)	
Marital Status, n (%)			1

	No	2(33.33)	63(40.65)	
	Yes	4(66.67)	92(59.35)	
Routine Exercise, n (%)				1
	No	2(50.00)	65(44.52)	
	Yes	2(50.00)	81(55.48)	
Alcohol Use, n (%)				0.23
	No	5(83.33)	85(54.84)	
	Yes	1(16.67)	70(45.16)	
Caffeine Use, n (%)				0.59
	No	0(0.00)	26(17.11)	
	Yes	6(100.00)	126(82.89)	
Race, n (%)				0.07
	Black	2(33.33)	110(70.97)	
	Other	4(66.67)	45(29.03)	
AMH (ng/mL), median (Q1-Q3)		3.85 (3.06-3.85)	2.48 (1.33-4.94)	0.59
Length of Infertility, years, median(Q_1-Q_3)		0.83 (0.04-1.08)	1.75 (0.67-4.00)	0.04
NAT2 Acetylator Status, n (%)				0.1
	Rapid	6(100.00)	85(64.39)	
	Slow	0(0.00)	47(35.61)	
Pregnant, n (%)				0.05
	No	7(100.00)	98(63.23)	
	Yes	0(0.00)	57(36.77)	
Cotinine mg/dL, median (Q_1-Q_3)		100 (27.25-100)	0.50 (0.00-100)	0.05
Cotinine mg/dL, n (%)				0.1
	0	1(16.67)	71(48.97)	
	<100	1(16.67)	37(25.52)	
	100	4(66.67)	37(25.52)	
Current, Active Smoker, n (%)				0.09
	No	3(42.86)	115(74.19)	
	Yes	4(57.14)	40(25.81)	

Self-reported SHS Exposure, n (%)				0.12
	Low	1(14.29)	78(50.32)	
	High	6(85.71)	77(49.68)	

CURRICULUM VITAE

T'shura Ali

EDUCATION

2016 - Present Doctor of Philosophy (PhD) in Public Health Science, Epidemiology
University of Louisville, Louisville, KY

Dissertation: "Association between Tobacco Smoke Exposure and Fertility-Related Outcomes among Females Seeking Fertility Treatment, and Interaction with N-Acetyltransferase 2 (NAT2)"
Dissertation Chair: Kira C. Taylor, Ph.D.

2013 - 2015 Master of Public Health (MPH), Epidemiology
University of Louisville, Louisville, KY

Practicum: "Global Health Initiative: A Bridge to Overcome the Barriers between Cultural Competency and Refugee Medical Care"
Practicum Advisor: Richard Kerber, Ph.D.

2007-2011 Bachelor of Arts, Biochemistry and Molecular Biology
Bellarmine University, Louisville, KY

Research: The anti-cancerous effects of Reishi mushroom on lung cancer proliferation in the female cell line H1793
Advisor: Johann Lau, Ph.D.

CERTIFICATION & TRAINING

May 2015 Certification in Public Health (CPH)
National Board of Public Health Examiners

RESEARCH EXPERIENCE

August 2016 - September 2019 — University of Louisville, Louisville, KY
Graduate Research Assistant
Louisville Tobacco Smoke Exposure, Genetic Susceptibility and Infertility (LOUSSI) study

- Recruit eligible women seeking fertility counselling/treatment
- Administer questionnaire
- Extract data from medical records
- Biological specimen collection
- DNA extraction from urine specimen
- Cotinine assay
- DNA genotyping using PCR
- Data entry
- Re-contacting participants
- Develop a master dataset with time-varying covariates
- Data cleaning and management
- Data analysis – Multivariable modelling (Survival Analysis and Logistic Regression)

March 2015 - May 2016 — University of Louisville, Louisville KY
Division of Infectious Disease - Clinical Research Associate

- Data Collection
- Database (REDCap) Entry
- Quality Control
- Follow-up of participants by telephone
- Lab bench work assistant

August 2010 - April 2011 — Bellarmine University, Louisville, KY
Undergraduate Research Assistant

- Western blot analysis of cell cycle regulatory proteins from lung cancer cells treated with Ganoderma lucidum
- Cell plating, gel electrophoresis and western blot analysis

TEACHING EXPERIENCE

August 2016 – December 2019 University of Louisville, Louisville, KY
Graduate Teaching Assistant

Courses:

- Introduction to Epidemiology (PHEP 501) - Fall 2016
- Foundations of Global Maternal Child and Health (PHEP 615) – Spring 2018
- Statistical Foundations for Epidemiology (PHEP 621) – Fall 2017, Fall 2018, Fall 2019
- Advanced Epidemiological Methods II (PHEP 701) – Fall 2018, Fall 2019
- Epidemiologic Methods and Concepts for Public Health (PHEP 441) – Spring 2019

- Assist faculty members with classroom instruction, exams, and other miscellaneous projects
- Tutor or mentor students
- Prepare presentations for lectures
- Deliver lectures
- Hand out assignments and grade papers
- Record grades and inform students of their final grades
- Meet with students during office hours
- Lead discussion sections

GUEST LECTURES

"Fertility and Fecundability" – University of Louisville School of Public Health and Information Sciences

- PHEP 615 : Foundations of Global MCH (Spring 2018)
- PHEP 624 : Methods in Reproductive Perinatal Epidemiology (Fall 2018)
- PHEP 615 : Foundations of Global MCH (Spring 2019)
- PHEP 624 : Methods in Reproductive Perinatal Epidemiology (Fall 2019)
- PHEP 615 : Foundations of Global MCH (Spring 2020)

WORK EXPERIENCE

August 2007 - December 2010 Bellarmine University, Louisville, KY
Help Desk, Work Study

- Set up equipment for events
- Troubleshoot students' devices
- Install and maintain printers, faxes, projectors, phones
- Print identification cards
- Answer phones, file documents, photocopy, assist students

June 2011- May 2013 — PhysAssist Scribes, Fort Worth, TX
Emergency Department Scribe – Audubon and Norton Downtown Hospital, Louisville KY

- Provide real-time charting for emergency room physicians
- Record patients' histories and chief complaints
- Transcribe physical exams
- Record diagnostic test results
- Prepare plans for follow-up care

Certified Scribe Trainer- Audubon Hospital, Louisville KY

- Train potential Emergency Department scribes at the Emergency Department
- Write evaluations on scribe trainees

Scribe Ambassador – Louisville Region

- Attend job fairs to recruit potential scribe candidates
- Develop the PhysAssist Scribe brand
- Develop key contacts
- Network with potential scribes
- Advertise to student groups, facilities and pre-health staff

January 2013 - March 2013 — Norton Healthcare, Louisville KY
EPIC Super User

- Train and assist physicians, nurses and hospital employees using EPIC electronic medical records system in the Emergency Department and Internal Medicine

VOLUNTEER EXPERIENCE

- Executive Advisory Committee - The Healing Place, Louisville, KY. 2016 - 2017. Plan, organize and carry out events such as 86 Addiction, the Healthcare Classic 5K, and Hope Classic Golf Scramble to raise money for the non-profit organization

- Community engagement – The Green Heart Project, Louisville, KY. Summer 2019. Interact with community members from the Green Heart study area to create a feedback loop between researchers and the community

SKILLS & TECHNIQUES

- R
- SAS
- SPSS
- REDCap
- Geographical Information Systems (GIS) Mapping
- OpenEpi
- EPIC (EMR) charting
- Microsoft PowerPoint
- Microsoft Excel
- Microsoft Word
- Genotyping using PCR
- Pipetting
- Gel Electrophoresis
- Maintaining Cell Cultures
- Proper Microscope Use
- Western Blot Analysis
- PCR
- General Laboratory Safety Training

AWARDS

- Doctoral Dissertation Completion Award. University of Louisville School of Public Health and Information Sciences. (Spring 2020)
- 2nd place for the Louisville Women in Medicine and Science Research Award at Research Louisville!. (October 2015)
- 3rd place in the Undergraduate Research Competition (Health Sciences) at Kentucky Academy of Science. (November 2010)
- Bellarmine University Dean's List (4 semesters: Fall 2007- Fall 2010)
- Presidential Achievement Scholarship Award (August 2007-May 2011)

PUBLICATIONS

1. Zolj, S., Smith, M. P., Goines, J. C., **Ali, T. S**., Huff, M. O., Robinson, D. L., & Lau, J. M. (2018). Antiproliferative effects of a triterpene-enriched extract from lingzhi or reishi medicinal mushroom, ganoderma lucidum (agaricomycetes), on human lung cancer cells. *International journal of medicinal mushrooms*, 20(12).

ORAL PRESENTATIONS

1. **Ali T'shura SA** and Lau JM. The anti-cancerous effects of Reishi mushroom on lung cancer proliferation in the female cell line H1793. Association of Southeastern Biologists, Huntsville, AL. April 14-16, 2011.

2. **Ali T'shura SA** and Lau JM. Western blot analysis of cell cycle regulatory proteins from lung cancer cells treated with Ganoderma lucidum., Math, Engineering, and Science Conference (MESCON), Evansville, IN. March 26, 2011.

POSTER PRESENTATIONS

1. Meredith Cahill, Gabrielle Farley, **T'Shura Ali**, Lindsey Wood, Kira Taylor, Henry Bohler, Islamiat Oladipupo. The association between polycystic ovarian syndrome and the probability of conception in women undergoing fertility counseling. Epidemiology and Population Health and Urogynecology. Research Louisville! Louisville, KY. September 2019.

2. **T'shura Ali**, Islamiat Oladipupo, Henry Bohler, Kelly Pagidas, MD, David W. Hein, Sashia Torres, Jasmine Chiang, Yelena Dondik, Adrienne Gentry, Merry Lynn Mann, and Kira Taylor. Childhood Secondhand Tobacco Smoke Exposure and Ovarian Reserve among Females Seeking Fertility Care, and Interaction with N- Acetyltransferase 2(NAT2) Genotype. American College of Epidemiology (ACE). Cincinnati, OH. September 2018.

3. Islamiat Oladipupo, **T'shura Ali**, Henry Bohler, FACOG, Kelly Pagidas, Jasmine Chiang, Yelena Dondik, Adrienne Gentry, Merry Lynn Mann, and Kira Taylor. Prevalence and Validity of Self-Reported Smoking Among Females Seeking Fertility Treatment. American College of Epidemiology (ACE). Cincinnati, OH. September 2018.

4. Islamiat Oladipupo, **T'shura Ali**, Henry Bohler, Kelly Pagidas, David W. Hein, Merry Lynn Mann, Adrienne Gentry, Yelena Dondik, Jasmine Chiang, and Kira C. Taylor. Association between smoking and ovarian reserve among females seeking fertility treatment, and interaction with N-Acetyltransferase 2(NAT2) genotype. Society for Perinatal Epidemiological Research (SPER). Baltimore, MD. June 2018.

5. **Ali, T'shura SA**, J Arnouk, K Lohano, L Binford, B Guinn, A Raghuram, R Nakamatsu, F Fernandez and F Arnold. Clinical outcomes of male vs. female patients hospitalized with community-acquired pneumonia (CAP). Infectious Diseases. Research Louisville! Louisville, KY. October 2015.

6. **Ali T'shura SA**, R Bosson, A Fuentes, KR Contreras, M Khaleefah, LR Hernandez and R Carrico. 2014. Global Health Initiative: Understanding Barriers to Primary

Care for Refugees from the Perspective of the Primary Care Provider. Research! Louisville. Louisville, KY. October 2014.

7. Robinson, DL, S Zolj, JC Goines, MP Smith, **TSA Ali**, MO Huff and JM Lau. 2013. Chemopreventative influence of Ganoderma lucidum extracts on male and female lung cancer cells. 99th annual meeting of the Kentucky Academy of Science, Morehead State University, Morehead, KY, November 8-9, 2013.

8. Zolj S, Pawley MD, **Ali TSA**, Goines JC, Robinson DL and Lau JM. 2011. Effect of extracts from reishi mushroom (Ganoderma lucidum) on lung cancer cell proliferation. Botanical Society of America. St. Louis MO.

9. **Ali T'shura SA**. and Lau JM. 2011. The anti-cancerous effects of Reishi mushroom on lung cancer proliferation in the female cell line H1793.Annual Celebration of Undergraduate Research at Bellarmine University, Louisville, KY. April 12.

10. **Ali T'shura SA**. and Lau JM. 2010. Western blot analysis of cell cycle proteins from lung cancer cells treated with Ganoderma lucidum. Kentucky Academy of Science, 96th Annual Meeting, Bowling Green, KY. Nov 13.

REFERENCES

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