ABSTRACT

Title of Thesis: THE EFFECTS OF FEMALE SEX HORMONES ON THE RELATIONSHIP BETWEEN PARA-OCCUPATIONAL EXPOSURE TO AGRICULTURAL WORK AND URINARY BLADDER CANCER AMONG EGYPTIAN WOMEN

India C. James
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Thesis Directed By: Olivia Carter-Pokras, Ph.D.
Department of Epidemiology and Biostatistics

Well-established risk factors such as tobacco smoke, occupational exposure, and *Schistosoma haematobium* infections do not fully explain the lower risk of bladder cancer for women compared to men. Recently, an association was reported between women who were married to agricultural workers and bladder cancer risk. We investigated whether proxies of estrogen exposure (age at first childbirth, number of babies delivered, and age at menopause) act as effect modifiers on the association of para-occupational exposure to agricultural work and bladder cancer among Egyptian women. Data from a multi-center Egyptian case-control study of 222 cases and 471 controls were used to examine the association. No evidence of effect modification was observed for these proxies. Later age at first childbirth may be an independent risk factor for this malignancy (AOR= 1.63; 95% CI 1.17, 2.27). Future studies can help identify sensitive sub-populations of Egyptian women who are more likely to develop bladder cancer.

THE EFFECTS OF FEMALE SEX HORMONES ON THE RELATIONSHIP BETWEEN PARA-OCCUPATIONAL EXPOSURE TO AGRICULTURAL WORK AND URINARY BLADDER CANCER AMONG EGYPTIAN WOMEN

By

India C. James

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Advisory Committee:
Professor Olivia Carter-Pokras, Chair
Professor Sania Amr
Professor Xin He
Professor Lesliam Quiros Alcala

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AOR	Adjusted Odds Ratio
CI	Confidence Interval
CIE	Change in Estimate
OR	Crude Odds Ratio
ETS	Environmental Tobacco Smoke
SCC	Squamous Cell Carcinoma
SD	Standard Deviation
SH	Schistosoma haematobium
UC	Urothelial Cell Carcinoma

Chapter I: Introduction

1.1.Background

1.1.1 Urinary Bladder Cancer & Known Risk Factors

Urinary bladder cancer is the ninth most common malignancy in the world, with an estimated 430,000 new cases diagnosed in 2012 (Antoni et al., 2017). The two most prominent histological types of bladder cancer are urothelial cell carcinoma (UC) and squamous cell carcinoma (SCC). Approximately, 90% of all cases of bladder cancer in the United States and other industrialized countries are of UC type (Theodorescu & Ehdaie, 2014); whereas, cases of SCC type are frequently observed in countries where *Shistosoma hematobium* (SH) infection is endemic (Othman & Soliman, 2015). Adenocarcinoma, an uncommon histologic variant of bladder cancer, accounts for approximately 2% of all bladder malignancies in the United States (Dadhania et al., 2015; Jacobo et al., 1977; Mostofi, 1968).

Depending on geographical location, the incidence of SCC and UC can vary because of increased exposures to distinct etiological risk factors and environmental influences (Parkin, Bray, Ferlay,& Pisani, 2005). Chronic infections with SH are associated with SCC in developing countries, particularly in Africa and the Middle East (Othman & Soliman, 2015). SH is a trematode, which induces chronic inflammation of the bladder that potentially leads to cell transformation. In Egypt, where schistosomiasis was endemic, 68% of bladder cancer cases were histologically identified as SCC prior to the country's governmental mass campaign to eradicate SH (Bedwani et al., 1997; El-Bolkainy et al. 1981; Mostafa et al. 1999). After the 1980s, SCC cases have substantially decreased to now represent 30-35% of urinary bladder cancer cases (Felix et al., 2008). However, the cumulative incidence of bladder cancer in Egypt has not substantially decreased, but rather a shift from SCC to UC has been reported (Felix et al., 2008).

Cigarette smoke accounts for roughly 45% of bladder cancers diagnosed (Hu, Sidransky, & Ahrendt, 2002). Tobacco smoke contains a variety of chemicals (e.g. aromatic amines, 2-naphthylamine, 4-aminobiphenyl, and benzidine (Letašiová et al., 2012). Some of these chemicals possess the ability to generate reactive oxygen species (ROS) (Berg JM & JL Tymoczko, 2002), which can damage cellular proteins and DNA and give rise to transformed cells. The growth of these cells has been shown to contribute to many diseases including bladder cancer (Gupta et al., 2012).

An extensive body of scientific literature has considered active cigarette smoking to be causally associated with bladder cancer (Office on Smoking and Health, 2004). Over 30 case-control studies (Zeegers et al., 2000) and a smaller number of prospective cohort studies (Doll et al., 2005; Jee, Samet et al., 2004) provided evidence for an association between cigarette smoking and bladder cancer. Epidemiologic studies have also consistently reported a two to threefold increased risk of bladder cancer among regular smokers compared to nonsmokers (Silverman DT et al., 2006; *Tobacco Smoke and Involuntary Smoking*, 2004).

Environmental tobacco smoke (ETS), known as secondhand smoke, was investigated as a possible contributor to bladder cancer. Non-smokers, who are exposed to involuntary smoke, were found to have elevated concentrations of carcinogenic N-nitroso compounds (Hecht, 2002) in their urine. However, epidemiological studies did not provide sufficient evidence between the risk of bladder cancer and exposures to environmental tobacco smoke (Alberg et al., 2007, 2007; Kabat, Dieck, & Wynder, 1986; Sandler DP, Wilcox AJ, & Horney LF, 1984).

Another well-known risk factor bladder cancer is occupational exposure to chemicals. Approximately 18% of all bladder cancer cases are attributed to occupational exposures (Letašiová et al., 2012). Certain industrial and agricultural chemicals have been linked to bladder cancer risk (Letašiová et al., 2012). Occupational exposures to chemicals are often observed among agricultural workers in open fields, greenhouse, and workers in pesticide industry (Damalas & Eleftherohorinos, 2011; Døssing & Skinhøj, 1985) .

Agricultural workers are exposed to a wide variety of potentially carcinogenic agents. These agents include pesticides, insecticides, herbicides fertilizers, animal virus, and numerous other microbes. Indeed, some pesticides are possible carcinogens (Alavanja et al., 2005; Dich, Zahm, Hanberg, & Adami, 1997), but only a few pesticides are associated with increased risk of bladder cancer (Koutros et al., 2016; Silverman, et al., 2016; Letasiovia et al., 2012; Viel, et al., 1995).

1.1.2 Urinary Bladder Cancer in Egypt

The incidence of bladder cancer in Egypt is relatively high, with 13.1 cases per 100,000 person-years in 2012 (Ferlay, J., 2014). The malignancy is still more commonly diagnosed in men than in women (Horstmann et al. 2008; Zheng et al. 2012). In 2012, the age standardized incidence rate (SIR) for this malignancy was 21.8 per 100,000 person-years for Egyptian men, and 5.6 per 100,000 person years for Egyptian women (Ferlay et al. 2014).

The distribution of tobacco smoking and exposure to occupational toxins, primary risk factors of bladder cancer, vary greatly between Egyptian men and women (Labib et al., 2007; Assaad R & Krafft, 2015). The use of tobacco products among Egyptian women is relatively low. In a 2007 survey, 22% to 47% of adult Egyptian men reported smoking, while only two to seven percent of adult Egyptian women reported smoking (Labib et al., 2007). The low participation of women in the Egyptian labor force also challenges the explanation that occupational toxins are a key risk factor for bladder cancer among women. According to the 2012 Egypt Labor Market Panel Survey, female labor force participation was recorded at 24% while male participation was 79% (Assaad R & Krafft, 2015). The 4:1 male to female ratio for bladder cancer persisted after the country's campaigns for antismoking and SH eradication (Wolpert et al., 2010). Thus, the well-established bladder cancer risk factors of tobacco smoke and occupational toxins only partially explain the observed disproportionately higher incidence rate of bladder cancer in men compared to women in Egypt.

1.1.3 Para-occupational Exposure to Agricultural Work

In the United States and Europe, ecological and other observational epidemiological studies have examined para-occupational exposures to pesticides and other agricultural chemicals in farming households (Alavanja et al., 2005; Ocaña-Riola, et al. 2004). Unlike occupational exposures, para-occupational exposure to agricultural work, also commonly referred to as "take-home exposure," focuses on the pesticide or chemical residues that are brought into the household through the clothing, shoes, hands, and tools of the agricultural worker (Strong et al. 2009).

The take-home exposure pathway provides a plausible rationale for the observed bladder cancer risk among women. Indeed, in a recent study, Jackson et al. found that married women, who reported their husband or head of household (H) to be an agricultural worker, had significantly higher odds of having bladder cancer (AOR=1.54, 95% CI 1.09, 2.18) than those who reported other occupations (Jackson, St George, Loffredo, & Amr, 2016). These findings suggest that the magnitude of exposure to agricultural work among women is somewhat related to their close contact with the workers and potential household cleaning duties (Jackson et al., 2016).

The performance of household duties highlights one of the pathways postulated for para-occupational exposures to agricultural chemicals (Deziel et al., 2015). Compared to the general population, agricultural workers are consistently found to be at higher risk of exposure to pesticides and other human carcinogens

(Alavanja et al., 2005; Ocaña-Riola et al., 2004). Through household cleaning duties, women are potentially exposed to agricultural chemicals when performing domestic responsibilities (e.g., cleaning the house and washing clothes), (Jackson et al, 2016). A longitudinal study by the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) reported further evidence of indirect transmission of chemical exposures in women who performed laundry of agricultural worker clothes (Eskenazi et al. 2003). Researchers reported 2-24% higher concentrations of serum dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH), both of which are pesticides, among women who washed agricultural worker clothes as compared to those who did not (Eskenazi et al., 2003).

The duration of domestic work among Egyptian women is "almost three times higher for young married women than their single counterparts" (Assaad R & Krafft, 2015). Therefore, it is plausible that women married to agricultural workers are more exposed than unmarried women to pesticides, biological agents, and other agricultural chemicals.

1.2 Estrogens and Bladder Cancer

A lower risk of bladder cancer was noted among Egyptian women who had menopause at the age of 45 or older compared to women who had early menopause (< 45 years) (Wolpert, 2010). The role of sex steroid hormones in the development and progression of bladder cancer was documented in animal studies (Zhang, 2013). Estrogens have been shown to inhibit bladder cancer, while androgens are documented to promote its progression (Chiu et al. 2016). Under the same dosing conditions, male mice developed bladder cancer, on average, 63 days earlier than female mice (Zhang, 2013).

The effect of sex steroid hormones on bladder tissues has also been well documented during pregnancy and menopause, where significant changes in the levels of sex steroid hormones occur (McGrath et al. 2006; Ntzeros et al. 2015). During these stages, the bladder can undergo changes in structure, function, histology, and gene expression (Ntzeros et al., 2015). Exogenous hormone use, and several factors identified as proxies for sex steroid hormones exposures have been investigated in other studies -- "parity, age at menarche, age at first pregnancy, and age at menopause" (McGrath et al., 2006). Both older age at first pregnancy and early age at menopause have been used as proxies for low lifetime estrogen exposure and were found to be significantly associated with an increased risk of bladder cancer among postmenopausal women (Kabat et al., 2013; Wolpert et al., 2010). However, other studies did not find similar results (Huang et al., 2009; Prizment, Anderson, Harlow, & Folsom, 2007a).

1.2.1 Age at first childbirth

Having the first child at an early age may serve as a protective factor in bladder cancer risk, because during pregnancy there is a surge in estrogens, where its serum concentrations increase approximately 100 fold (Yen S, 1994). As seen in animal studies, the increased duration and levels of exposure to estrogens can inhibit the growth and progression of bladder cancer (Chiu et al., 2016). Age at menarche can be related to the age at first childbirth (Sandler DP et al., 1984), and thus, increased exposure to estrogens from early onset of regular menstrual cycle may protect against tumor initiation (Chiu et al., 2016). Several epidemiological studies have reported mixed results on the relationship between age at first childbirth and bladder cancer (Chiu et al., 2016, Weibull, Eloranta, Altman, Johansson, & Lambe, 2013) . The majority of these studies were conducted in the United States, Taiwan, and some European countries. The use of different populations and outcomes could explain the mixed results observed. The Taiwan study by Chiu et al. (2016) used a population-based cohort and examined the association between parity and bladder cancer mortality; they found a significant increase in mortality risk with increasing age at first childbirth. The study also showed that, after adjustment for age, parity, and educational level, women who had their first childbirth delivered after the age of 26 years had an HR of 2.30 (95% CI:1.21,4.39); as compared to those who gave birth for the first time at age < 23 years. In contrast, a Swedish population-based cohort study found the incidence rate of bladder cancer to decrease with increasing age at first childbirth (Weibull et al., 2013).

1.2.2 Number of babies delivered

Surveys of cancer incidence and mortality suggest that bladder cancer risk may decrease with increasing parity (Cantor et al. 1992; Green 1988; Plesko et al., 1985). Theoretically, the greater the number of childbirth, the higher the overall cumulative exposure to sex hormones (Chiu et al., 2016). The number of babies delivered is also related to the number of pregnancies because of significant hormonal changes during gestation. An earlier case-control study by Cantor (1992) in Iowa found that parous women (i.e., women who have given birth to live born children) were at a decreased risk of bladder cancer as compared to nulliparous women (OR=0.67; 95% CI: 0.44, 1.00), after adjustment for age, tobacco use, and previous bladder infection. Another case-control study in 2010 found that women with greater number of babies delivered (> 6) are at lower risk of bladder cancer, compared to those who had fewer babies (Wolpert et al., 2010). However, other epidemiologic studies have not reported statistically significant associations between parity and risk of bladder malignancy (Chiu et al., 2016; Cantwell et al., 2006)

1.2.3 Age at menopause

With menopause, monthly estrogen surge ceases. Decreased levels of estrogen are associated with bladder dysfunction in postmenopausal women (Huang et al., 2009). Some symptoms of bladder dysfunction are recognized as recurrent urinary tract infections and incontinence (Hextall, 2000). Several epidemiologic studies have revealed a strong association between elevated bladder cancer risk and recurring urinary tract infections (Foxman, 2010; Vermeulen et al., 2015). Early age at menopause has been speculated to increase bladder cancer risk due to (1) decrease in estrogen exposure, and (2) recurrence of urinary tract infections and concurrent inflammation (McGrath et al., 2006). Compared with older age at menopause (age ≥ 50 years), younger age at menopause (age ≤ 45 years) was associated with a statistically significant increase in bladder cancer risk (Incidence Rate Ratio=1.63; 95% CI: 1.20, 2.23) (McGrath et al., 2006). In contrast, an Italian case-control study found no significant association between menopausal status and age at menopause and bladder cancer risk (Pelucchi et al. 2002). However, the Italian case-control study had only nine premenopausal cases, limiting their statistical power to detect any significant associations.

1.3 Gaps in the Knowledge

To our knowledge, there is no study on the relationship between para-occupational exposure to agricultural work, bladder cancer risk, and the role of estrogen exposure among women. In Egypt, the traditional risk factors for bladder cancer, (e.g., tobacco smoke and agricultural work) are highly prevalent among men but not women (Mandil et al. 2012). Lifetime exposures to estrogen may serve as a protective factor for bladder cancer and provide an explanation for the lower risk of bladder cancer in women, as compared to men. However, previous epidemiological studies have reported inconsistent findings on the role of estrogen in bladder cancer research (Cantwell, Lacey, Schairer, Scatzkin, & Michaud, 2006). In this study, we seek to investigate whether estrogen is an effect modifier of the association between bladder cancer risk and para-occupational exposure to agricultural work among a sample of women who are not exposed to the well-established risk factors for this malignancy.

The rationale for conducting the present study is to explore why women have a lower risk of bladder cancer than men. The exact mechanism underlying this difference is not completely understood, despite the fact that excess rates of bladder cancer among men can be partially explained by higher smoking rates and more exposures to occupational carcinogens than in women.

1.4 Research question/ Specific Aims

This study's objective is to use reproductive health variables (e.g. age at first childbirth, number of babies delivered, and age at menopause) as proxies for estrogen exposure, and assess whether they modify the relationship between para-occupational exposure to agricultural work and bladder cancer risk among Egyptian women who are married to agricultural workers. The specific aims and hypotheses for this study are:

Specific Aim 1: to assess whether age at first childbirth acts as an effect modifier on the association between para-occupational exposure to agricultural work and urinary bladder cancer among Egyptian women.

Research Hypothesis (1): Among women exposed to para-occupational agricultural work, the odds of having bladder cancer will be higher for those who reported having their first child at an age >18 years than for those who had their first child at an earlier age (≤18 years old).

Specific Aim 2: to assess whether number of babies delivered acts as an effect modifier on the association between para-occupational exposure to agricultural work and urinary bladder cancer among Egyptian women.

Research Hypothesis (2): Among Egyptian women exposed to para-occupational agricultural work, the odds of having bladder cancer will be higher for those who reported having delivered fewer babies (≤6) than for those who reported having delivered a higher number of babies (>6).

Specific Aim 3: to assess whether age at menopause acts as an effect modifier on the association between para-occupational exposure to agricultural work and urinary bladder cancer among Egyptian women.

Research Hypothesis (3): Among women exposed to para-occupational agricultural work, the odds of having bladder cancer will be higher for those who reported menopause at an early age (≤ 45 years) than for those who reported menopause at a later age >45 years).

Chapter II: Methods

2.1 Study Design

The present study uses a subset of de-identified dataset from a multi-center case-control study, "Gender differences in bladder cancer risk factors in Egypt", conducted between 2006 and 2014. Details of the parent study were previously described (Zheng et al, 2012).

2.2 Study population

Bladder cancer cases were recruited from three referral cancer centers in Egypt: the National Cancer Institute in Cairo, the Minia Oncology Center in Minia, and the South Egypt Cancer Institute in Assiut (Zheng et al., 2012). Each of the referral cancer centers serves a specific area in Egypt including the metropolitan area of Cairo, the surrounding areas in northern Egypt, and the upper and lower region of South Egypt (Zheng et al., 2012). Eligible cases were identified as: (1) adults ages

19-80 years, (2) diagnosed within 12 months with presumed bladder cancer, and (3) self-identified as able to participate in the interview (Zheng et al., 2012). Further, eligible cases included men and women. For each bladder cancer case, one of the two study pathologists confirmed the histopathology of primary urinary bladder cancer (UC, SCC, adenocarcinoma, others including undifferentiated). Participants with prior history of other cancer were excluded.

Controls were randomly selected from the population and frequency matched to the cases by gender, age (5-year interval) and governorate of residence (Zheng et al., 2012). For each governorate, the number of cases who were already recruited and who resided in that governorate determined the number of controls and their ages. Two methods were applied to recruit controls: (1) random sampling of households, and (2) random sampling of family health records (Zheng et al., 2012). Each method of recruitment used two primary sampling methods for selecting controls. For the random sampling of households, streets were randomly selected in the designated villages. Within the selected street, a systematic method of random sampling was applied to approach residents of the household (Zheng et al., 2012).

If the house occupants did not match the gender and age range-controls, the recruitment team would move to the next house on the selected street.

For the random sampling of family health records, primary care health units function as the sampling frame for selecting healthy controls in the districts (also known as neighborhood or village within the governorate). Most administrative districts in Egypt have a government-subsidized medical unit, where residents can receive healthcare (Zheng et al., 2012). As a result, these units contain medical records of families living in the specific district. Within the primary care health unit, a systematic method of random sampling was applied to examine family health records of potentially matched controls. Prospective participants were approached at home by the study's recruiter who explained the purpose of the study and offered participation (Zheng et al., 2012). The study received permission from the Egyptian Ministry of Health to access family health records in the primary care health units (Zheng et al., 2012).

2.2.1 Analytic Sample

For this study, the analytic sample was restricted to female controls and cases with either UC, SCC, or adenocarcinoma, who were married, did not work outside of the home, self-identified as non-smokers, and had data on the occupation of the husband (n = 693). For the analysis with age at menopause, the analytic sample was further restricted to women who had reported to have stopped menstruation (n = 476).

2.2.2 Data Collection

In the parent study, cases were recruited from three referral cancer centers as described in the previous section. Informed consent was obtained from the study participants by trained interviewers who administered the same structured questionnaire to both cases and controls. Data collection on sociodemographic characteristics included: age, residence location, marital status, education, working outside the home and type of occupation, smoking history, and exposure to environmental tobacco smoke (Zheng et al., 2012). Medical history of schistosomiasis was also documented. Furthermore, information on environmental exposures to pesticides at home or at work was collected as well as the occupation of the husband. The reproductive history questions for the women included, but were not limited to age at first childbirth, menopausal status, age at menopause, and number of babies delivered.

2.3 Variable Definitions

2.3.1 Independent Variable

Para-occupational exposure to agricultural work, which is defined as the female participant's husband occupation, was the main exposure assessed in the present study. The categories of occupations for the spouse were originally listed as agricultural worker, manual laborer, mechanic, clerical worker, student, merchant or trade worker, or shepherd (Jackson et al., 2016). However, for our analysis, the occupation of the husbands was categorized as agricultural worker versus others. The recoding of the two new categories was represented by "1" for agricultural workers and "0" for others.

2.3.2 Dependent Variable

The dependent variable for this thesis was primary bladder cancer.
One of the two study pathologists confirmed the histopathology of primary urinary bladder cancer and ascertained the case to be UC, SCC, adenocarcinoma, or undifferentiated carcinoma (Zheng et al., 2012). Cancer of other organs that metastasized to the bladder were excluded from the study. In the present analysis, the dependent variable was recoded as "1" for bladder cancer cases that included UC, SCC or adenocarcinoma only, and "0" for controls.

2.3.3. Estrogen Exposure Variables

The three reproductive health variables: age at first childbirth, number of babies delivered, and age at menopause were used as proxies for estrogen exposure. In the parent study, the reproductive health variables were recorded as continuous variables. For our analysis, each reproductive health variable was assessed for its distribution among the controls. We used empirical data to determine the cut-off points for these variables to dichotomize each of the continuous variables. Further, evidence from previous literature supports our established cut off points for each estrogen exposure proxy (Huang et al., 2009; Jackson et al., 2016; Prizment et al., 2007)

Based on the median distribution for age at menopause, the cut off point among the controls was 45 years. Prior to the assessment of age at menopause, we examined and verified whether the married women reached the first criteria: stopped menstruation. Out of the sample of 693 married women, only 476 women reported to have stopped menstruation. Thus, participants who reached menopause at or before age 45 were classified as having early menopause and coded 1; whereas those who reached menopause after the age of 45 were classified as having late menopause and coded 0.

According to the median distribution, the cut-off point for the number of babies delivered was six among controls. Hence, the number of babies delivered was divided into two categories: women who reported having delivered fewer babies (≤ 6) were coded as 1 and those who reported having delivered a higher number of babies (> 6) were coded as 0.

Lastly, the cut off point for the age at first childbirth was 18 years, which was determined by the median distribution. Therefore, the age at first childbirth was categorized as an early age (≤ 18 years of age) and coded 0, and a later age (>18 years) and coded 1.

2.3.4. Covariates: Potential Confounders

The rationale for selecting these covariates as potential confounders derives from two sources: (1) traditional criteria for identifying confounders and (2) evidence from other studies. The three traditional conditions for classifying a confounder are (a) the third factor must be a cause or risk factor of the disease; (b) the third factor must be positively or negatively associated with the exposure; and (c) the third factor must not be an intermediate step in the causal pathway (McNamee, 2003).

The following covariates were assessed as potential confounders of the association between para-occupational exposure to agricultural work and urinary bladder cancer: history of schistosomiasis, education, pesticide use at home, and secondhand smoke exposure. The history of schistosomiasis and pesticide use at home were recorded based on the "no/yes" responses of the study's participants. Participants who responded "don't know" regarding their history of schistosomiasis or pesticide use at home were coded as missing.

Education included the following categories: none, primary school/kottab, high school/technical school, and college/university. In the present analysis, education was categorized as "no school" versus "school". The variable was recoded to indicate “0” for no school and “1” for primary school/kottab, high school/technical school, or college/university. Secondhand smoke exposure was assessed by the criteria of three variables: anybody smoking at home, husband ever smoked at home, and exposure to smoke other than at home. A participant was classified as exposed to secondhand smoke if they answered, “yes” to any of the three questions and they were coded 1.

Age of the participant and place of residence (south versus north and urban versus rural), as the matching variables for this case-control study, were included in the final analyses.

2.3.5. Covariates: Potential Effect Modifiers

We assessed whether either of the following covariates: age at first childbirth, number of babies delivered, and age at menopause, modified the association between para-occupational exposure to agricultural work and urinary bladder cancer. Unlike confounders, effect modification highlights the biological or natural phenomenon that occurs when the magnitude of the primary exposure on the outcome differs by the level of the third variable (Knol, 2012).

2.4 Statistical Analysis

2.4.1. Descriptive Statistics

Variables were compared between cases and controls by using Chi-square test and Student’s T test for categorical and continuous variables, respectively.

2.4.2. Unadjusted Analysis

Association between bladder cancer and each of the independent variables was examined using logistic regression to obtain crude odds ratio (OR) and respective 95% confidence interval (CI).

2.4.3. Adjusted Analysis: Multivariable logistic regression

Logistic regression modeling estimated the associations between the independent covariates and the cancer status. The model included husband's occupation as agricultural work, and frequency-matching variables (age and place of residence). Results were reported as adjusted odds ratios (AORs), and 95% confidence intervals (CI). All statistical analyses were performed using SAS version 9.3. (The SAS Institute).

2.4.4. Interaction Terms

To investigate effect modification of estrogen exposure on the association between para-occupational exposure to agricultural work and urinary bladder cancer, we used interaction terms in the multivariable models. Each reproductive health variable was assessed separately in the multivariable model before and after inclusion of its interaction term with the main predictor. Prior to assessing interaction terms, the proxies for estrogen exposure (e.g. age at first child, number of babies delivered, and age at menopause) were included in separate multivariable regression models to investigate the association of estrogen exposure with the outcome. Interaction terms were considered statistically significant if the p-value was less than 5% ($p<0.05$).

2.4.5. Confounders

We used the 10% change-in-estimate criterion (CIE) to identify confounders (e.g. schistosomiasis, education, and pesticide use at home) (Lee & Burstyn, 2016). Education was the only significant confounder included in the final model.

2.5 Human Subjects

The present study consists of a secondary analysis of de-identified data from a multi-center case-control study conducted in Egypt between 2006 and 2014. The parent study was approved by the institutional review boards of Georgetown University and the University of Maryland, Baltimore, by the ethics committees of the three referral centers, and the ministry of health in Egypt. Prior to participating in the parent study, written or witnessed informed human consent was obtained from all of the study participants (Zheng et al., 2012). If participants were unable to read or write, the witness informed human consent was obtained by having the written consent read to participants and witnessed by somebody else. Prior to data analysis, this research was determined to not be human subjects research by the Institutional Review Board of the University of Maryland, College Park (See Appendix).

Chapter III: Results

3.1 Sociodemographic Characteristics

Table 3.1 shows sociodemographic characteristics of the study sample, which consisted of more controls (n=471) than cases (n=222). Husband occupation reported as an agricultural worker, education, pesticide use at home, schistosomiasis, age at first childbirth, and menstrual status were significantly different by case status ($p < 0.01$). The majority of the cases and controls had no formal education (91.4% and 77.6%, respectively). Controls were more likely (36.5%) than cases (21.6%) to report use of pesticides at home. Cases were more likely than controls to report that their husband was an agricultural worker, history of schistosomiasis, and had their first childbirth at a later age (>18).

Table 3.1: Sociodemographic characteristics of married Egyptian women bladder cancer cases and controls who do not work outside the home or smoke

Characteristics	Cases n=222 (%)	Controls n=471 (%)	p-value[a]
Husband occupation			
Other	109 (49.1)	290 (61.6)	<0.01
Agricultural worker	113 (50.9)	181 (38.4)	
Education			
No formal education	203 (91.4)	364 (77.6)	<0.01
Formal education	19 (8.6)	105 (22.4)	
Pesticide use at home			
No	174 (78.4)	298 (63.5)	<0.01
Yes	48 (21.6)	171 (36.5)	
History of Schistosomiasis			
No	148 (72.6)	394 (87.4)	<0.01
Yes	56 (27.5)	57 (12.6)	
Age at first childbirth (years)			
Earlier age ≤ 18	105 (47.3)	273 (58.0)	<0.01
Later age > 18	117 (52.7)	198 (42.0)	
Number of babies delivered			
> 6	82 (36.9)	188 (39.9)	
≤ 6	140 (63.1)	283 (60.1)	0.45
Menstruation Stopped			
No	39 (17.6)	177 (37.7)	<0.01
Yes	183 (82.4)	293 (62.3)	
Age at Menopause (years) (cases n=183 & controls n=293)			
Later age > 45	92 (50.3)	167 (57.0)	
Earlier age ≤ 45	91 (49.7)	126 (43.0)	0.16

[a]p-value calculated using chi square test for categorical variables and Student's t test for continuous

3.2 Unadjusted Associations

For the bivariate analysis, we found statistically significant associations between occupation of the husband as an agricultural worker, education, pesticide use at home, history of schistosomiasis, age at first childbirth, menstrual status, and the odds of having bladder cancer. As shown in Table 3.2, having a husband who is an agricultural worker (OR=1.66; 95% CI: 1.20, 2.30) and having a history of schistosomiasis (OR=2.62; 95% CI: 1.72, 3.96), were positively associated with bladder cancer risk. Formal education (OR=0.32; 95% CI: 0.19, 0.55) and pesticide use at home (OR=0.48; 95 CI%: 0.33, 0.70) were inversely associated with bladder cancer risk.

For the proxies of estrogen exposure, we found that later age at first childbirth (>18 years) (OR=1.54; 95% CI: 1.11, 2.11), and achieving menopause (OR=2.83; 95% CI: 1.91, 4.20) were significantly associated with an increased risk of bladder cancer.

Table 3.2: Crude Associations between study variables and bladder cancer among married Egyptian women who do not work outside the home or smoke

Characteristics	Odds Ratio (95% CI) (n=693)
Husband occupation	
Other	REF
Agricultural worker	1.66 (1.20, 2.29)
Education	
No formal education	REF
Education	0.32 (0.19, 0.55)
Pesticide use at home	
No	REF
Yes	0.48 (0.33, 0.70)
History of Schistosomiasis	
No	REF
Yes	2.62 (1.72, 3.96)
Age at first childbirth (years)	
Earlier age ≤ 18	REF
Later age > 18	1.54 (1.11, 2.11)
Menstruation Stopped	
No	REF
Yes	2.83 (1.91, 4.20)
Age at Menopause (years) (among women who had menopause (n= 476)	
Later age > 45	REF
Earlier age ≤ 45	1.31 (0.90, 1.90)

CI: confidence interval

3.3 Multivariable Logistic Model

In the multivariable logistic model, in addition to the main independent variable (husband occupation), the following covariates were included: age and governorate of residence (e.g. north vs. south and urban vs. rural). Although, these covariates were not significantly associated with bladder cancer risk, they were included in the adjusted model because they were the matching variables.

The results of the multivariable logistic regression models, before inclusion of any of the estrogen proxy variable are shown in Tables 3.3 and 3.4. After adjustment for the covariates cited above, the AOR for having bladder cancer among women who were married to agricultural workers was statistically significant (AOR=1.67; 95% CI: 1.20, 2.33).

Table 3.3: Adjusted odds ratio and 95% confidence intervals of the associations between living in households with agricultural workers and bladder cancer among nonsmoking married Egyptian women

Predictor variable	Odds Ratio (95% CI) n=693
*Husband occupation	
Other	REF
Agricultural worker	1.67 (1.20, 2.33)

*Adjusted for age, north vs. south, and rural vs. urban

As shown in Table 3.4, the odds ratio of the main effect among postmenopausal women (AOR=1.58; 95% CI: 1.08, 2.33) was similar to that of the total sample, as seen in Table 3.3.

Table 3.4: Adjusted odds ratio and 95% confidence intervals of the associations between living in households with agricultural workers and bladder cancer among post-menopausal nonsmoking married Egyptian women

Predictor variable	Odds Ratio (95% CI) n=476
*Husband occupation	
Other	REF
Agricultural worker	1.58 (1.08, 2.33)

*Adjusted for age, north vs. south, and urban vs. rural

3.4 Effect modification: Interaction Terms

The presence of effect modification was determined by the interaction term approach. The multivariable model was fitted separately for each reproductive health variable and its interaction terms with the main predictor. All of the adjusted models included matching variables: age and governorate of residence (e.g. north vs. south, and urban vs. rural).

The interaction terms (age at first childbirth by husband occupation, babies delivered by husband occupation, and age at menopause by husband occupation) were not significant. Thus, no evidence of multiplicative interaction was observed between husband occupation as an agricultural worker and the three estrogen exposures in relation to bladder cancer risk.

3.5 Examining role of estrogen proxies

In Table 3.5, Models 1, 2, and 3 show the AOR for the husband occupation in the presence of age at first childbirth, number of babies delivered, and age at menopause, respectively. In Model 1, later age at first childbirth (>18 years) was significantly associated with bladder cancer risk (AOR=1.63; 95% CI: 1.17, 2.27). Number of babies delivered (Model 2) and age at menopause (Model 3) were not significantly associated with bladder cancer, although the ORs were elevated (AOR=1.26; 95% CI: 0.87, 1.82; AOR=1.12; 95% CI: 0.74, 1.69 respectively).

Table 3.5: Multivariable regression models of the associations between living in households with agricultural workers and bladder cancer risk among nonsmoking married Egyptian women, by age at first childbirth, number of babies delivered, and age at menopause

Predictor variable	Odds Ratio (95% CI) n=693
Model 1	
Husband occupation	
Other	REF
Agricultural worker	1.51 (1.08, 2.13)
Age at first childbirth	
Earlier age	REF
Later age	1.63 (1.17, 2.27)
Model 2	
Husband occupation	
Other	REF
Agricultural worker	1.54 (0.95, 1.94)
Babies delivered	
> 6 babies	REF
≤ 6 babies	1.26 (0.87, 1.82)
Model 3*	
Husband occupation	
Other	REF
agricultural worker	1.50 (0.87, 1.97)
Age at menopause	
> 45	REF
≤ 45	1.12 (0.74, 1.69)

*only women who reached menopause were included in this model (n=476)
All models were adjusted for age, education, north vs. south, and urban vs. rural

3.6 Confounders

To determine whether schistosomiasis, education, and pesticide use at home had a confounding effect on the association between living in a household with an agricultural worker and bladder cancer risk, we compared the estimates of the association among married Egyptian women, before (Tables 3.3) and after including the well-known risk factors of bladder cancer in Table 3.6.

The main adjusted estimate (AOR= 1.67; 95% CI: 1.20, 2.33) in Table 3.3 decreased after including schistosomiasis (AOR=1.56; 95% CI: 1.10, 2.21) and pesticide use at home (AOR= 1.58; 95% CI: 1.13, 2.21), as seen in Model 2 and Model 3 of Table 3.6. These well-known risk factors produced changes in the adjusted model that were less than 10% of the CIE (7.69% for schistosomiasis, & 6.50% for pesticide use at home). Thus, schistosomiasis and pesticide use at home do not have significant confounding effect on the association. A change in the AOR (1.67; 95% CI: 1.20, 2.33) in Table 3.3 was observed after including education in Model 4 of Table 3.6 (AOR=1.50, 95% CI: 1.07, 2.10). Therefore, education did result in a change in the CIE greater than 10% (11.24% for education). Hence, education was considered a protective factor for this study.

Table 3.6: Multivariable regression models of the associations between living in households with agricultural workers and bladder cancer risk among nonsmoking married Egyptian women, by schistosomiasis, education, and pesticide use at home

Predictor variable	Odds Ratio (95% CI)
Model 1	
Husband occupation	
Other	REF
Agricultural worker	1.67 (1.20, 2.33)
Model 2	
Husband occupation	
Other	REF
Agricultural worker	1.56 (1.10, 2.21)
Schistosomiasis	
No	REF
Yes	2.55 (1.67, 3.90)
Model 3	
Husband occupation	
Other	REF
Agricultural worker	1.58 (1.13, 2.21)
Pesticide use at home	
No	REF
Yes	0.50 (0.34, 0.73)
Model 4	
Husband occupation	
Other	REF
Agricultural worker	1.50 (1.07, 2.10)
Education	
No	REF
Yes	0.35 (0.20, 0.60)

All models were adjusted for age, north vs. south, and urban vs. rural

Chapter VI: Discussion

4.1 Summary

We observed a positive and statistically significant association between para-occupational exposure to agricultural work and having bladder cancer among non-smoking married Egyptian women (AOR=1.50; 95% CI: 1.07, 2.10), after adjustment for age, education, and governorate of residence (north vs. south, and urban vs. rural).

Age at first childbirth was significantly associated with bladder cancer risk in the adjusted model (AOR=1.63; 95% CI: 1.17, 2.27). The latter finding is consistent with findings from a previously published study and the hypothesis that early age at first childbirth may function as a protective factor against bladder cancer risk (Chiu et al., 2016).

We also found that menopause was significantly associated with increased bladder cancer risk (OR=2.83; 95%CI: 1.91, 4.20) among married Egyptian women. However, age at menopause was not significantly associated with the disease (OR=1.31; 95% CI: 1.31, 1.90). Several studies have documented that early age at menopause was associated with an elevated risk of bladder cancer (McGrath et al., 2006; Prizment, Anderson, Harlow, & Folsom, 2007), while other studies have found no association (Davis-Dao et al., 2011; Huang et al., 2009; Pelucchi et al., 2002). Menopause can occur naturally or be induced surgically (oophorectomy) or medically.

In a reproducibility and validity study of self-reported age at menopause, den Tonkelaar suggested that the effect of the latter may be underestimated. For self reported data, the precision of reporting age at menopause tends to decrease with increasing number of years since the final menstrual period (den Tonkelaar, 1997). Thus, women who had early menopause may subsequently report an older age at menopause (Appiah et al., 2016). And a more recent study showed the reliability of age at menopause is higher in women who underwent surgical than natural menopause, and it decreased with time since menopause (Lucas, Azevedo, & Barros, 2008). In our study, women were asked about their age at, but not the type of menopause. Furthermore, the analytic sample size for age at menopause was smaller (n=476) compared to the study sample (n=693). Perhaps, we were unable to demonstrate effect modification by age at menopause due to the small sample size and limited power. Therefore, it is possible that the lack of association observed between bladder cancer and early age at menopause is the result of misclassification of self-reported age at menopause and smaller sample size.

The history of schistosomiasis was also found to be statistically significantly associated with bladder cancer ($p < 0.05$). Schistosomiasis plays a significant role in the causation of urinary bladder cancer. Studies have found that SCC is more common in areas with a high prevalence of schistosomiasis compared to areas of low prevalence (Rambau, Chalya, & Jackson, 2013). However, schistosomiasis did not meet or exceed the 10% CIE for a confounding variable in our study. The other well-known risk factors, education and pesticide use at home, were inversely associated with bladder cancer risk ($p < 0.05$). Our findings suggest that education and pesticide use at home are protective factors against bladder cancer risk.

Previous findings reported a lower risk of bladder cancer among subjects who were educated, compared with those who were illiterate (Vizcaino A. et al, 1994).

Education can function as another indicator of socioeconomic status that closely relates to cancer (Jacobs et al., 2012). A higher educational level has been found to be associated with lower cancer risk in a number of studies (Jacobs Bruce, 2012; Mouw et al., 2008). One study found considerably higher incidence rates of bladder cancer among women of low socioeconomic status (Jacobs Bruce, 2012). In a study, Donnelly (2013) found a strong positive relationship between bladder cancer and socioeconomic status among women. Therefore, it is plausible that educated women have more access to health care (e.g. medical offices, cancer screening, and health resources) than women with lower socioeconomic status.

Interestingly, a high correlation between pesticide use and education was observed in the results of 2006 study of UK residents (Steer C et al., 2006), where residents who had high incomes, better education, and non-manual occupations were more likely than those with low income, little education and manual occupations to use pesticides in the home and garden (Steer C et al., 2006). It is possible that the type, quantity, and mode of application of pesticide use in the home are different from the chemical sprays used in agricultural fields. Thus, the exposure might be different, which can potentially explain the different direction of the association between pesticide use at home and bladder cancer risk.

4.2 Strengths & Limitations

The present study has a number of strengths that included the overall study design, selection of the controls, adjustment for well-known risk factors of bladder cancer, and the use of multiple centers for recruitment.

The case-control study design provided information on potential risk factors of bladder cancer in a cost- and time-effective manner. The use of population-based controls and frequency-matched variables helped minimize selection bias and potential confounding by age and location of residence. The adjustment for several well-known risk factors of bladder cancer also contributed to minimize selection bias and potential confounding. We were able to reduce the confounding effect of smoking, the most well-known risk factor of bladder cancer, by restricting the sample to only non-smokers. Potential misclassification of outcome was reduced as case status was confirmed by at least one of the two study pathologists using a standardized case definition of bladder cancer (Zheng et al., 2012). Finally, the use of multiple cancer centers to recruit cases from several regions in Egypt helps improve generalizability.

Despite these strengths, the study has several limitations. The final analytic sample (n=693) had some missing data for the following covariates: schistosomiasis (n=38), education (n=2), pesticide use at home (n=2), and menstrual status (n=1). Although missing data points were excluded from the analysis, there is still a possibility of misclassification of schistosomiasis. A total of 8% of the cases and 5% of the controls reported unknown history of schistosomiasis. Also, the analytic sample size for age at menopause was smaller (n=473) compared to the study sample. Hence, there is a possibility of lower power for age at menopause due to the smaller sample size.

Most of the reproductive health information was self-reported, and thus subject to potential recall bias from the study participants; however, there is no reason to believe that such recall was differential between cases and controls. Indeed, the women in our study were unlikely to be aware of prior research findings on the potential associations of bladder cancer with the reproductive health variables. Nonetheless, considering previous studies of reliability showing that self-reported age at menopause varies with time since menopause (Lucas, Azevedo, & Barros, 2008; den Tonkelaar 1997), it is possible that age at menopause in our study was not accurate.

No biological specimens were collected to confirm the presence of pesticides or other chemicals residues potentially associated with bladder cancer and used in farming. The study did not collect information on the women's home proximity to a farm. Several studies have proven that adverse health risks are associated with living in areas with high density of livestock farms (Radon et al., 2007; Schulzr Anja et al., 2011). Livestock farms are known to harbor a variety of compounds including microbial compounds, particulate matter, and volatile organic compounds (Dungan, 2010). Populations residing in agricultural areas may also be exposed to pesticides through drift, which derives from agricultural fields in proximity to their home (Ward et al., 2006). Thus, the study cannot eliminate potential interfering chemical compounds or pesticides transported from the farm or fields to the home of the women.

Another limitation of our study is the absence of information on the specific types of pesticides used among the Egyptian agricultural workers. In a previous study conducted by Ezzat et al (2005), a list of potential pesticides to which agricultural workers are exposed to in Egypt was described. Hence, we can assume that agricultural workers are exposed to a mixture of chemicals.

Finally, there is always the possibility of residual confounding as in any epidemiologic study. Although, we excluded smokers from the analysis, it is possible that women participants may have been hesitant to report smoking.

4.3 Future Studies

Our findings suggest that estrogen exposure proxies (age at first childbirth, number of babies delivered, and age at menopause) might not be the most effective protective factors on bladder cancer risk among Egyptian women who were married to agricultural workers. Researchers might obtain a better understanding of pesticide exposure among these women by combining survey questions with external measurements.

Future studies can incorporate external measurements to estimate the amount of pesticides and pesticide residues in the household of farmers. The use of external measurements including personal air samplers and pesticide applicators can determine the maximum amount of exposure to pesticides, which are affecting the women. The collection of urine samples for women married to agricultural worker can also assist in confirming the presence of pesticides. The combination of questionnaire research with external measurements will further assist in identifying and estimating concentration of pesticides encountered by the wives and husbands.

Researchers can also conduct a more detailed evaluation of pesticide use in Egypt using shop receipts as an alternative approach to direct measurements. This more detailed evaluation can provide useful information not only on the type of pesticide but also on the changes of pesticide exposure over time. The average consumption of pesticides in Egypt has gradually declined during the 1970s-1990s (Mansour, 2004).

In addition to the massive evaluation of pesticide use, future studies should include a larger sample of women who use birth control and hormone replacement therapy (HRT) to better assist in determining the cumulative exposure to estrogen. For our study, there was a very small number of women who used birth control and HRT. To obtain a more diverse profile on female reproductive factors, a larger sample of women who used hormonal contraceptive and experienced hormone therapy are needed. For this purpose, future studies are needed to investigate the exact mechanism of estrogen exposure in bladder cancer risk.

4.4. Implication for practice

The overall aim of our research is to raise awareness about secondhand exposure to agricultural pesticides and estrogen exposures among a specific population of women. To reduce secondhand exposure, it is imperative for the women to understand the importance of safe handling practices with clothing and tools of their agricultural working spouse. Future practices can integrate well-established cleaning techniques of items that were exposed to pesticides.

Some examples include (1) wash clothes of the agricultural worker separately from other members of the family and (2) store tools of the agricultural worker outside of the home. These alternative techniques can assist in minimizing the exposure to agricultural pesticides. In addition to the exposure of pesticides, we were mainly interested in exploring effect modification of estrogen exposure on the association between paraoccupational exposure to agricultural work and bladder cancer risk.

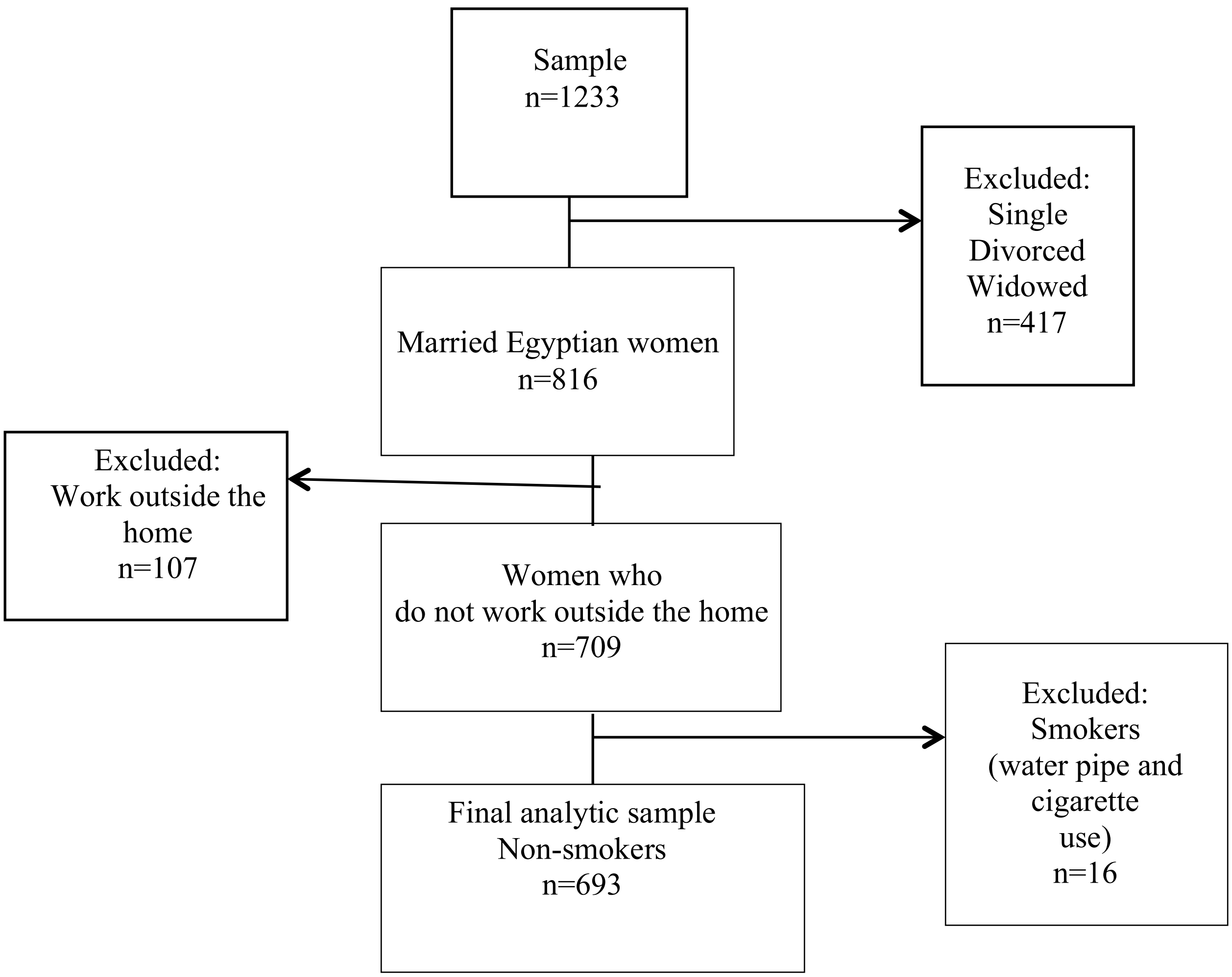
From a public health perspective, if estrogen exposures were found to modify the association, the results would help identify sensitive sub-populations of Egyptian women who are more likely to develop bladder cancer. However, additional research is needed to explore our research question.

4.5 Conclusion

In summary, none of the estrogen exposure proxies (age at first childbirth, number of babies delivered, and age at menopause) appear to be potential effect modifier of the association between para-occupational exposure to agricultural work and bladder cancer risk among married women in Egypt. A later age at first childbirth (older than 18 years) was an independent factor for bladder cancer risk among these women. The combination of future studies and use of external measurement for pesticide residues may achieve a better understanding of the role of estrogen exposure on the development of bladder cancer risk among women who are married to agricultural workers. Findings from future research may also assist in the development and tailoring of new urinary bladder cancer prevention programs for these newly identified at-risk populations.

Appendices

Figure 1: Exclusions for bladder cancer study



Attachment#1: Confirmation of IRB approval from University of Maryland, College Park, MD

UNIVERSITY OF MARYLAND

INSTITUTIONAL REVIEW BOARD

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DATE: September 7, 2017

TO: Olivia Carter-Pokras, PhD
FROM: University of Maryland College Park (UMCP) IRB

PROJECT TITLE: [1125066-1] The Effects of Female Sex Hormones On The Relationship Between Para-occupational Exposure To Agricultural Work and Urinary Bladder Cancer Among Egyptian Women

SUBMISSION TYPE: New Project

ACTION: DETERMINATION OF NOT HUMAN SUBJECT RESEARCH
DECISION DATE: September 7, 2017

Thank you for your submission of New Project materials for this project. The University of Maryland College Park (UMCP) IRB has determined this project does not meet the definition of human subject research under the purview of the IRB according to federal regulations.

We will retain a copy of this correspondence within our records.

If you have any questions, please contact the IRB Office at 301-405-4212 or irb@umd.edu. Please include your project title and reference number in all correspondence with this committee.

This letter has been electronically signed in accordance with all applicable regulations, and a copy is retained within University of Maryland College Park (UMCP) IRB's records.

- 1 -

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