# Consequences of female night shift work for fertility, assisted conception and fetal development

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## Abstract

It is estimated that 1 in 7 women in paid employment in Australia are working at night, on either permanent or rotating shift rosters. Night shift work can disrupt circadian rhythms, contributing to cardiometabolic disturbance, psychosocial stress, and could potentially affect human reproductive health. This thesis investigated whether female night shift workers were more likely to require fertility treatment to achieve a first birth and whether this reflected specific reproductive health problems. The thesis then examined whether this combination of patient and treatment factors contributed to adverse perinatal outcomes, specifically congenital urogenital anomalies, in the first births of night shift workers when compared to non-shift workers.

An initial step entailed a review of the literature concerning night shift work and female fertility, miscarriage and perinatal outcomes, resulting in a published manuscript focusing on fertility (including time to pregnancy, menstrual irregularity and endometriosis) and miscarriage. This manuscript represents a multidisciplinary project in which a complex literature is critiqued and summarised. It also provided an overview of current clinical guidance and policies in place internationally.

Australian research on shift work has been constrained by the absence of a tool to assess this exposure on a large scale. Accordingly, a shift work job-exposure matrix specific to occupations in Australia was developed using established methods. The job-exposure matrix was applied to a large population-based cohort of births produced via linkage of routine perinatal registries with fertility clinic data. These included the South Australian Perinatal Statistics Collection, the South Australian Birth Defects Register and data from the two clinics registered to provide fertility treatment in South Australia between 1986–2002. This allowed identification of primiparous women with probable exposure to light at night

during night shift work, forming the basis for two subsequent studies of reproductive function and treatment outcomes.

One study considered the use of fertility treatment among night shift workers. The analysis indicated that a higher proportion of women in occupations likely to involve night shift conceived their first birth with fertility treatment, compared to their unexposed counterparts in paid employment (OR = 1.25, 95% CI 1.09–1.43). However, this was attenuated when adjusted for age (OR = 1.10, 95% CI 0.95–1.26). Among those who accessed treatment, night shift workers were more likely to be diagnosed with menstrual irregularity (OR = 1.42, 95% CI 1.05–1.91) and endometriosis (OR = 1.34, 95% CI 1.00–1.80).

A second study examined the associations of night shift work, subfertility and fertility treatment with urogenital anomalies occurring in first births. This outcome was pre-specified based on plausible mechanisms linking circadian rhythms to perturbed maternal endocrinology and subsequent fetal exposures in utero. Results indicated that singleton births to primiparous night shift workers, conceived using fertility treatment, were more likely to have a urogenital anomaly, compared to those of non-shift workers who conceived using fertility treatment (OR = 1.80, 95% CI 0.94–3.46). The effect was greater among multiple births (OR = 2.94, 95% CI 1.26-6.85). This finding was not related to differences in the type of fertility treatment received by night shift workers compared to other women who did not work night shift. The outcome was also specific to fertility treatment, and did not extend to naturally conceived singleton or multiple births to night shift workers in the general population, for which there was no significant association. Further analysis indicated an ordering of risk, whereby the greatest risk of urogenital anomalies occurred among births that were jointly exposed to maternal night shift work and fertility treatment (OR for singletons = 2.11, 95% CI 1.17–3.79), an additive interaction.

This thesis represents the first research to investigate the use of fertility treatment among female night shift workers and whether this contributes to congenital anomalies in offspring. The finding that night shift workers were more likely to conceive their first birth using fertility treatment is potentially a consequence of a

higher prevalence of menstrual irregularities and endometriosis. Alternatively it may reflect psychosocial effects of night shift work, or a combination of these factors, on women's age of first childbearing. A subgroup of women who undertake night shift work may be most susceptible to the effects of circadian disruption on their fertility, either directly or through exacerbation of underlying fertility problems, which contribute to adverse outcomes for offspring conceived using fertility treatment.

Future research concerning night shift work, infertility, and recourse to fertility treatment would benefit from inclusion of all women who undergo fertility treatment, regardless of whether a birth was achieved. Despite this limitation, the depth and breadth of information in this large population-based cohort has enabled the first steps towards identifying requirements for fertility treatment by night shift workers and demonstrated a combined impact of patient and treatment factors on fetal development.

## Thesis declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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**Publications arising from this thesis**

Fernandez RC, Peters S, Carey RN, Davies MJ, Fritschi L. Assessment of exposure to shiftwork mechanisms in the general population: the development of a new job-exposure matrix. Occupational and Environmental Medicine 2014; 71: 723–729

Fernandez RC, Marino JL, Varcoe TJ, Davis S, Moran LJ, Rumbold A, Brown HM, Whitrow MJ, Davies MJ, Moore VM. Fixed or rotating night shift work undertaken by women: implications for fertility and miscarriage. Seminars in Reproductive Medicine 2016; 34(2): 74–82.

Fernandez RC, Moore VM, Marino JL, Davies MJ. Night shift among women: is it associated with fertility treatment to conceive a first birth? (To be submitted)

Fernandez RC, Moore VM, Willson KJ, Davies MJ. Does maternal shift work contribute to the excess of urogenital defects after fertility treatment? (To be submitted)

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Dated:                   _____

**Conference presentations arising from this thesis**

1.  Fernandez RC, Marino JL, Varcoe TJ, Davis S, Moran LJ, Rumbold AR, Brown HM, Whitrow MJ, Davies MJ, Moore VM. *Shift work is a public health issue: impacts on fertility in women* (Oral presentation). 15th World Congress on Public Health, Melbourne, 3-7 April 2017.

2.  **Fernandez RC**, Willson KJ, Moore, VM & Davies MJ. *Application of a shift work job-exposure matrix to investigate occupational differences in women's accessing of fertility treatment and infertility aetiology* (Oral presentation). 25th Epidemiology in Occupational Health Conference (EPICOH), Barcelona, 4–8 September 2016.

3.  **Fernandez RC**, Willson KJ, Moore, VM & Davies MJ. *Are female shift workers more likely to require treatment for infertility?* (Oral presentation). 2015 Robinson Research Institute Symposium, Adelaide, 4 November 2015.

4.  **Fernandez RC**, Willson KJ, Moore, VM & Davies MJ. *Maternal shift work and the risk of urogenital defects in offspring conceived using infertility treatment* (E-poster). Annual Scientific Meeting of the Fertility Society of Australia (FSA 2015), Canberra, 13–16 September 2015.

5.  **Fernandez RC**, Willson KJ, Moore, VM & Davies MJ. *Maternal shift work and the risk of urogenital defects in offspring conceived using infertility treatment* (Poster and invited oral presentation). DOHaD, Melbourne, 17–19 April 2015.

6.  **Fernandez RC**, Willson KJ, Moore, VM & Davies MJ. *Does maternal shift work increase the risk of urogenital birth defects?* (Poster). 2014 Robinson Research Institute Symposium, Adelaide, 6 November 2014.

7.  **Fernandez RC**, Davies MJ, Willson KJ & Moore, VM. *Methodological limitations associated with the use of administrative datasets in reproductive epidemiology: an example showing the impact of missing data on risk estimates for birth defects* (Poster). Florey International Postgraduate Research Conference, Adelaide, 25 September 2014.

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## Glossary of acronyms

| | |
|---|---|
| AFT | autologous fresh transfer |
| AIHW | Australian Institute of Health and Welfare |
| ANZARD | Australia and New Zealand Assisted Reproduction Database |
| ART | assisted reproductive technologies |
| ASCO | Australian Standard Classification of Occupation |
| BESST | birth emphasising a successful singleton at term |
| BMI | body mass index |
| CI | confidence interval |
| DI | donor insemination |
| ET | embryo transfer |
| FET | frozen embryo transfer |
| FSH | follicle stimulating hormone |
| GIFT | gamete intrafallopian transfer |
| hCG | human chorionic gonadotrophin |
| HR | hazard ratio |
| IARC | International Agency for Research on Cancer |
| ICD | International Classification of Diseases |
| ICI | intracervical insemination |
| ICSI | intracytoplasmic sperm injection |
| ISCO | International Standard Classification of Occupation |
| IUI | intrauterine insemination |
| IVF | in vitro fertilisation |
| JEM | job-exposure matrix |
| LBW | low birth weight |
| NPESU | National Perinatal Epidemiology and Statistics Unit |
| OI | ovulation induction |
| OR | odds ratio |
| PCOS | polycystic ovary syndrome |
| PESA | percutaneous epididymal sperm aspiration |
| PGD | preimplantation genetic diagnosis |
| RERI | relative excess risk due to interaction |

| RR | relative risk |
|---|---|
| RTAC | Reproductive Technology Accreditation Committee |
| SA | South Australia |
| SCMC | sperm cervical mucus contact test |
| SCN | suprachiasmatic nucleus |
| SERM | selective estrogen receptor modulator |
| SGA | small for gestational age |
| TET | tubal embryo transfer |
| TTP | time to pregnancy |
| UK | United Kingdom |
| USA | United States of America |
| WHO | World Health Organization |
| ZIFT | zygote intrafallopian transfer |

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# 1  Introduction

## Introduction and outline

Shift work is an inevitable part of modern society and 16% (1.5 million) of Australians in paid employment are involved in shift work. Of the 14% of Australian women in paid employment who are engaged in shift work, 67% are of reproductive age (20–44 years).[1] The proportion of women employed in shift work is likely to grow as the population ages, and demand increases for workers in the service and care industries in which females workers predominate.[2, 3]

It is important to point out that not all shift work is alike in terms of the extent to which it creates a mismatch between biological and social functioning and the demands of industry and society. The impact of shift work, particularly night and rotating shifts, on human health occurs via disruptions of the normal timing of biological activities, including hormone secretion, metabolism, digestion and sleep. Such disruptions cause internal bodily functioning and environmental stimuli to become asynchronised, leading to both acute and chronic health effects including cardiovascular disease, metabolic disorders and cancer.[4] Women's involvement in night shift work is of particular concern because of the potential for the adverse metabolic and reproductive consequences of circadian disruption to impair fertility as well as the likelihood of a healthy pregnancy and birth.[4]

The overarching aim of this thesis is to investigate the impact of night shift work on female fertility and recourse to fertility treatment to achieve birth. In addition, the relative contributions of night shift work (a patient factor) and assisted conception (treatment factors) to congenital urogenital anomalies in the offspring are explored.

Despite growth in both shift work and uptake of treatment for infertility, this question has not been examined previously in detail. Further, there has been no previous investigation of the potential effects of maternal night shift work on fetal

urogenital development, even though there are plausible mechanisms linking circadian rhythms to perturbed maternal endocrinology and subsequent fetal exposures in utero. Urogenital anomalies are also among the most commonly diagnosed congenital anomalies, both in naturally conceived pregnancies and those resulting from assisted conception.

The thesis begins with a background chapter that introduces the topics of fertility, fertility treatment and perinatal outcomes. The thesis is then structured around four projects. Project one comprises an overview of the effects of night shift work on several reproductive outcomes among the general population including fertility and conception, pregnancy loss and selected perinatal outcomes including congenital anomalies, preterm birth and low birth weight. A portion of this review, that concerning fertility, conception and pregnancy loss, has been published as a stand-alone paper.

Project two considers exposure assessment and begins with a review of occupational exposure assessment methods used in epidemiological research. This section highlights the need for a method to infer night shift work from the relatively limited occupational data available in routine data collections, such as perinatal records. A job-exposure matrix (JEM), which is a cross-classification of job titles and occupational exposures, provides a useful approach in this situation. Although existing shift work JEMs exist, they have not been produced using Australia data and have not previously been applied to the Australian context. Project two therefore describes the development of a new JEM for use in the projects three and four. The development of the JEM provides the content of the second published article contributing to this thesis by publication.

In preface to projects three and four, an account of the construction of South Australian (SA) Birth Cohort is provided. Both projects draw on data from this cohort.

The third project concerns the possible effect of night shift work on fertility. Firstly, this study sought to examine the uptake of fertility treatment among female night shift workers. Secondly, whether specific diagnoses are more

common among these women compared to non-shift workers also receiving treatment. The results of this study are presented in manuscript format. These analyses (and those of project four) were restricted to primiparous women conceiving their first birth. This increases the likelihood that participants were employed in their designated usual occupation around the time of conception.

While perinatal outcomes including miscarriage, preterm birth and low birth weight have been the subject of systematic reviews and meta-analyses (at least in the general population),[5, 6] very few studies have considered the effect of maternal night shift work on congenital anomalies in offspring. There has been no previous investigation of how the combination of night shift work and subfertility contribute to the increased risk of congenital anomalies observed among medically assisted conceptions.[7] Of particular interest are urogenital anomalies, as there is evidence that the aetiology of some types of urogenital anomalies, such as hypospadias, may be influenced by hormonal balance in utero.[8] This gap in the literature provides the impetus for the fourth project, which considers the risk of urogenital anomalies among births to primiparous female night shift workers who conceived using fertility treatment. Comparison with the risk of urogenital anomalies among naturally conceived first births to night shift workers is made in an attempt to disentangle the contribution of night shift work, subfertility and fertility treatment. Finally, the manuscript for this study is presented, following a description of some special methodological issues that are relevant in reproductive and perinatal epidemiology.

**Statement of aims**

The purpose of this thesis is to advance knowledge in relation to the following research questions: (1) what is the impact of women's night shift work on fertility and fetal development outcomes, and (2) how do patient and treatment factors contribute to abnormal fetal development among pregnancies from assisted conception to female night shift workers?

The specific aims of this thesis are as follows.

Aim 1: To review the literature relating to the effects of night shift work on several reproductive outcomes including fertility and conception, pregnancy loss and selected perinatal outcomes including congenital anomalies, preterm birth and low birth weight.

Aim 2: To develop a tool that is relevant to the Australian context and appropriate for assessing shift work exposure in large collections of routine data.

Aim 3: To examine the uptake of fertility treatment among female night shift workers and whether specific conditions contributing to subfertility are more likely to occur among night shift workers.

Aim 4: To establish whether maternal night shift work is a patient factor that contributes to the increased risk of urogenital anomalies among ART births and to determine whether this is also related treatment type.

## 1.1 Extended background

**Reproductive health and assisted reproductive technologies (ART)**

Adverse reproductive outcomes can occur at several stages along the reproductive continuum, which spans from conception to embryonic and fetal development through to the perinatal period. This section describes the major adverse events along this continuum, providing the definition and epidemiology of these outcomes, as well as broad coverage of their causes. To allow comparison, the prevalence of each outcome is provided separately for the general population and the ART population. Infertility and congenital anomalies are covered in more detail as these are a major focus of this thesis.

**Infertility**

The International Committee for Monitoring Assisted Reproductive Technology and the World Health Organization define infertility as the inability to achieve a pregnancy after 12 or more months of regular unprotected sexual intercourse.[9] Fertility problems relating to conception are often measured using the time (in months) to pregnancy (TTP), which is how long it takes a couple to conceive, or fecundability, which is the cycle specific probability of conception.[10, 11] Infertility may also involve the inability to carry a pregnancy through to a live birth.[12]

The prevalence of infertility reported in the literature varies depending on whether the focus is on current or lifetime infertility. In a review of infertility in developed and developing countries, Boivin et al.[13] indicate that at any given time approximately 9% of couples in developed countries are currently experiencing infertility. Estimates of lifetime infertility (i.e. having ever experienced infertility) in Australia range from about 16–24% depending on how infertility is defined. In the National Fertility Study 2006, 17% of adults over 18 years reported a TTP longer than 12 months.[14] A similar figure (17.3%) was reported among women aged 28–33 years participating in the Australian Longitudinal Study on Women's

Health.[15] Using a more general definition, Marino et al.[16] found that 24% of women aged 30–32 years who had attempted to become pregnant, reported having trouble conceiving. Infertility was ranked 18th in the 20 leading causes of incident non-fatal burden of disease/disability in females in 2003.[17]

Among infertile couples, the problem is generally accepted to be related to male factors in 20% of cases, female factors in 30%, joint male and female in 40% of cases and unknown causes in 10% of cases.[18] Couples diagnosed with unexplained infertility usually return normal results to diagnostic tests of infertility including semen analysis, luteal phase assessment, postcoital testing, immunological testing and examinations for tubal, cervical and uterine abnormalities.[19]

Male infertility is often characterised by abnormal semen quality, that is, the semen contains dysfunctional sperm with reduced capacity for fertilisation. This includes, but is not limited to, reduced (or absent) sperm numbers, abnormal sperm motility and/or abnormal sperm morphology (form and structure).[20] In approximately 25% of cases, the cause of abnormal semen quality is idiopathic (i.e. due to an unknown cause).[12, 21] Known causes include problems with sperm transport from the testes to ejaculation, testicular failure and, less commonly, hypothalamic/pituitary failure.[12] Other factors that can impair male fertility include systemic disease such as autoimmune disorders, chemotherapy and lifestyle and environmental factors such as smoking, obesity and exposure to toxic chemicals.[12, 20]

Female infertility may be caused by a number of factors. Ovulatory dysfunction is the most commonly diagnosed cause of infertility in women. It accounts for 27% of cases where a diagnosis can be made.[21] Ovulatory disorders include menstrual cycle disturbances and hormonal disturbances involving hyper- or hypo-concentrations of reproductive hormones including FSH, estradiol and prolactin.[19] A common cause of ovulatory infertility is polycystic ovary syndrome (PCOS), which affects up to 20% of women of reproductive age. It is a complex condition with varied clinical features, including reproductive, metabolic and psychological sequelae.[22]

Tubal infertility is diagnosed in up to 20% of couples diagnosed with female factor infertility and involves damage to one or both of the fallopian tubes.[21] Common causes of tubal infertility include pelvic infection, such as pelvic inflammatory disease (often secondary to a sexually transmitted infection), and blockage caused by scar tissue following pelvic surgery.[23] Tubal damage may also be caused by endometriosis, which occurs up to 10% of women in the general population.[24] Endometriosis is an oestrogen-dependent, inflammatory disorder of complex aetiology, that is characterised by the presence of endometrial glands and stroma outside of the uterine cavity.[25]

Other causes of female infertility include cervical mucus defects, hormonal and autoimmune disorders.[26, 27] Also, as with males, lifestyle and environmental factors can impair fertility in females. For example, obesity is associated with hormonal and ovulatory disturbance.[26]

Advancing female age is strongly associated with declining fertility in both the general population and women undergoing fertility treatment. After the age of 35 years, female fertility and pregnancy rates decline significantly.[26] Advancing age also has a negative effect on male fertility, however, this decline is less dramatic compared to females, and the age at which males experience significant declines in fertility is less certain.[26, 28]

**Assisted reproductive technologies**

Impaired fertility is increasingly being overcome with the aid of fertility treatment, incusing assisted reproductive technologies (ART). Assisted reproductive technology is defined in Australia as 'the application of laboratory or clinical techniques to gametes and/or embryos for the purposes of reproduction'.[29] Other fertility treatments (not classified as ART) include treatment with drugs to induce ovulation and artificial insemination. It is estimated that at least 4.4% of children born in Australia are now conceived using fertility treatment.[30] This section defines ART within the Australian context, including the prevalence of

use and access arrangements, commonly used techniques and measures used to define successful treatment.

*ART in Australia*

In an Australian cohort study, 57% of women who had ever had difficulty conceiving sought medical assistance.[16] These findings were consistent with the average proportion of couples who seek assistance in resource-rich countries. Of those who sought medical assistance, 41% were treated with medication only and 20% went on to receive more invasive ART treatment.[16] In comparison, a UK study found that 26% of couples were offered for medication for ovulation induction as a first line treatment.[31]

Fertility treatment services have been subsidised under the Australian Medicare Benefits Scheme since 1990. Initially, couples were limited to six cycles; however this limit was removed in 2000.[32] Associated pharmaceutical costs are also subsidised by the Pharmaceutical Benefits Scheme. Currently, ART services are broadly accessible as more than 50% of the direct treatment costs are covered under these schemes and there are no restrictions to access based on age, number of treatment cycles or existing family size.[16, 33]

According to the Australian and New Zealand Assisted Reproduction Database (ANZARD), 73,598 ART treatment cycles were initiated in Australia and New Zealand in 2014. This represents 13.9 cycles per 1,000 Australian women of reproductive age (15–44 years). The majority (92.0%) of these ART cycles were conducted in Australia.[30] It is likely that these figures underestimate total treatment cycles as this database does not collect information about less intensive forms of fertility treatment such as intrauterine insemination and cycles involving ovulation induction only. In terms of diagnoses, 19.7% reported male factor infertility only, 30.8% reported female infertility only, 12.5% reported combined male and female infertility, 22.3% reported unexplained infertility and 14.7% did not indicate the cause of infertility.[30]

*ART treatment techniques*

This section provides an overview of the basic steps involved in a typical ART treatment cycle. These five steps also define the term *in vitro* fertilisation or IVF[12, 30]:

1. Ovulation induction (OI): pharmacological treatments are administered to a woman over a number of days to stimulate maturation of multiple oocytes (eggs). Treatment usually begins on day two or three of the woman's menstrual cycle (which may be induced if she is anovulatory).

2. Oocyte pick-up: mature oocytes are retrieved from ovarian follicles by aspiration. Aspiration is conducted under anaesthesia and involves the removal of oocytes and follicular fluid from the ovary using a needle attached to a suction device.[34]

3. Fertilisation: collected oocytes are incubated with sperm (collected from partner or donor) in the laboratory to allow fertilisation.

4. Embryo maturation: the fertilised oocyte is cultured for 2–3 days to form a cleavage embryo (6–8 cells) or 5–6 days to form a blastocyst (70–100 cells and presence of a fluid filled cavity).

5. Embryo transfer: one or more of the highest quality (see appendix 1 for description of embryo quality assessment) embryos are transferred into the uterus (2–3 days after oocyte retrieval and fertilisation or longer for blastocyst transfer) with the hope that pregnancy will be established.[12]

Variations of this basic IVF cycle are often employed in practice, depending on the aetiology of infertility and personal circumstances of couples.[30] Other fertility treatment methods that are most relevant to this thesis are described below. Supplementary information regarding other aspects of fertility and ART treatment methods can be found in appendix 1.

Intracytoplasmic sperm injection (ICSI) is a form of IVF whereby fertilisation occurs by the direct injection of a single spermatozoon into the oocyte cytoplasm.[9] This procedure is often used if there is failure of conventional IVF.[12] This procedure is also used to overcome male factor infertility that cannot be reversed surgically or medically.[21] In 2014, this procedure was used in 68% of ART cycles in which fertilisation was attempted.[30] There has been rapid growth in the use of ICSI to achieve fertilisation in both Australia and the United States, with current usage rates more than double that of 1996.[35, 36] This is mainly driven by increased use of ICSI for cases that do not involve male factor infertility.[35]

An ART treatment cycle may involve the use of gametes or embryos that have been cryopreserved. This involves the freezing or vitrification and storage of gametes, zygotes or embryos. Freezing involves traditional slow freezing, whereas vitrification is an ultra-rapid method of cryopreservation.[9] These gametes/embryos may be later used in frozen/thawed ART cycles. In Australia in 2014, 37.4% of ART cycles involved the use of frozen/thawed embryos.[30]

The use of a women's own oocytes/embryos in an ART treatment cycle is termed an autologous cycle. Women who are unable to use their own oocytes may undertake ART treatment using donated oocytes and/or embryos. A donation cycle refers to an ART treatment cycle that is initiated by a woman with the intention of donating her oocytes. In a recipient cycle, a woman receives oocytes/embryos that where donated by another woman. The donation of sperm is not considered a donation cycle. A couple may also donate their embryos to other couples if they are no longer required for their own ART treatment.[29] In 2014, just under 5% of total initiated treatment cycles in Australia were donor/recipient cycles.[30]

Artificial insemination involves placing partner or donor sperm in the woman's reproductive tract, in the hope that this will lead to fertilisation. This procedure is termed intracervical (ICI) or intrauterine (IUI) insemination depending on where the sperm is placed. This may be performed with or without ovulation induction. In 2014, 3,089 donor sperm insemination cycles were undertaken in fertility centres within Australia and New Zealand, and 12.8% resulted in a live birth.[30]

Data on the number of artificial insemination cycles using partner's sperm was not available.

Gamete intrafallopian transfer (GIFT) involves the laparoscopic transfer of the collected gametes (oocytes and sperm) into the fallopian tubes.[9, 34] Fertilisation therefore occurs *in vivo*. The use of this procedure has declined due to improvements in the effectiveness of IVF, and now accounts for only a small number of ART treatments.[12] In 2014, six GIFT cycles were conducted across Australia and New Zealand, none of which resulted in clinical pregnancy.[30]

*Measuring the success of ART treatment*

Many different measures are reported when communicating the effectiveness or success rate of ART treatment. These measures depend on the specific definitions of treatment success and treatment cycle. Some examples include the rate of fertilisation, rate of implantation, rate of pregnancy (hormonal or clinical) and the rate of live birth. Given that the primary goal of undertaking ART treatment is to produce a live baby, the rate of live birth is arguably the most relevant measure of treatment success from a patient, clinical and public health perspective.[12]

There has also been debate over what is the most appropriate denominator to use when calculating these rates. The early focus of clinicians was to provide these outcomes as a rate per cycle. This is complicated by variations in the definition of a 'cycle', and creates difficulties when comparing success rates across infertility clinics. This is because of differences in clinic specific policies regarding the reporting of initiated and cancelled treatment cycles.[37] There are now moves, driven by consumer groups and the Cochrane Collaboration, to measure success on a per woman basis. Therefore, a more appropriate definition of an ART cycle may be *an ovarian stimulation cycle initiated with the intent to apply ART*, which takes into account all women regardless of whether oocyte retrieval, fertilisation and embryo transfer stages are reached.[12]

Min et al.[38] recommend that ART programs should use the BESST (Birth Emphasising a Successful Singleton at Term) per cycle initiated approach, which

takes into account the health of the baby, to assess treatment success. This measure emphasises singleton births at term, as multiple pregnancies/births are known to increase the risk of delivery complications and may compromise the health of mothers and babies.[39, 40] In order to meet the goal of a healthy singleton birth, there has been a shift in Australian and New Zealand towards single embryo transfer. This is not the case for all countries, where different policies and reimbursement arrangements act as a disincentive for single embryo transfer.[41]

Use of BESST to evaluate ART success is not without limitations. The BESST outcome measure, while being of primary concern to patients, may not accurately reflect the performance of infertility clinics.[42] It overlooks the effect of clinic based policies on the number of embryos transferred and the transfer of frozen-thawed embryos,[37, 43] and does not consider the methods and intensity of ovarian stimulation, which may be important factors in the success of ART treatment.[44, 45] Lastly, adverse events occurring after pregnancy is established may be related to obstetric and perinatal care, rather than the performance of the infertility clinic or laboratory.[45]

**Miscarriage**

Pregnancy can be detected biochemically within the first few weeks of conception by testing urine or serum for a rise in human chorionic gonadotrophin (hCG).[46] Clinical pregnancy is then diagnosed when an embryonic sac(s) or fetal heart beat is detected by ultrasound scan at 6–7 weeks gestation.[9, 47] Miscarriage or spontaneous abortion is defined as the loss of a clinical pregnancy before 20 weeks gestational age.[9] The prevalence of spontaneous loss of clinically recognised pregnancies amongst the general population is 10–15% [48(p319)] however, this figure is greatly increased if losses before any diagnostic tests are included. It is estimated that more than 60% of pregnancies among the general population are lost before the end of the first trimester,[49] with the majority of losses occurring before the pregnancy is clinically recognised.[50] These early miscarriages are often not recognised by women as pregnancy losses.[51]

The prevalence of miscarriage is higher among pregnancies conceived using fertility treatment. In Australia in 2014, 21.2% of ART cycles that produced clinical pregnancy resulted in early pregnancy loss. This includes 19.4% lost due to miscarriage, 0.5% due to termination and 1.2% due to ectopic or heterotopic pregnancy.[30] After adjusting for maternal age, Wang et al.[52] found a 20% increase in the relative risk of miscarriage in ART pregnancies compared to the general population. However, compared to the general population, there is greater monitoring of early pregnancy in women undergoing fertility treatment. Therefore, the prevalence of early miscarriage among ART pregnancies may only appear higher compared to naturally conceived pregnancies due an increased likelihood of detection.

Chromosomal abnormalities are a major cause of early pregnancy loss, accounting for 50% of miscarriages.[53] Other causes include implantation of the embryo in an inhospitable endometrium, endocrine and autoimmune disorders such as diabetes, exposure to environmental toxins and tobacco smoking.[48] In women undergoing ART, age, smoking and the transfer of poor quality embryos have also been shown to significantly increase the risk of early pregnancy loss.[47, 54]

Recurrent miscarriage is defined as the loss of three or more consecutive pregnancies. The prevalence among couples in the general population who are trying to conceive is 1%.[55] Recurrent miscarriage may be caused by genetic abnormalities such as aneuploidy, structural abnormalities of the uterus, endocrine abnormalities such as insulin resistance, immune dysfunction and antiphospholipid syndrome.[55]

**Fetal death**

Pregnancy loss involving death of the fetus at or after 20 weeks gestational age is termed fetal death or stillbirth.[9] The rate of fetal death among the general population in Australia was 7.0 per 1,000 births in 2013.[56] Among Australian ART births in the same year, the rate of fetal death was 9 per 1,000 births.[57] Major causes of fetal death include chromosomal abnormalities (15–20%) and fetal structural abnormalities (35%). Other risk factors include some bacterial, viral and

parasitic infections, diabetes (diabetes mellitus and gestational diabetes), advanced maternal age, obesity and cord or placental complications.[58]

**Congenital anomalies**

Congenital anomalies, also known as congenital abnormalities or birth defects, include all structural, functional and genetic anomalies diagnosed in aborted fetuses, at birth or during the neonatal period.[9] Congenital anomalies diagnosed in childhood are also included in some registries, including the South Australian (SA) Birth Defects Register, which collects data on anomalies diagnosed in children up to five years of age.[59] Australian burden of disease data for children aged 0–14 years indicates that congenital conditions contribute to 12% of total disability-adjusted life years lost and 41% of fatal and 59% of non-fatal burden of disease.[17]

The most recent (2012) yearly estimate of the prevalence of congenital anomalies among the SA general population is 4.4% of total births (live births and stillbirths).[59] Figure 1 contains the prevalence (per 1,000 total births) of congenital anomalies occurring in SA by diagnostic category for the period 1986–2012. The most commonly reported congenital anomalies to the SA Birth Defects Register during this period were urogenital anomalies with a prevalence of 16.7 per 1,000 total births.[59]

**Figure 1:** Frequency of congenital anomalies notified to the South Australian Birth Defects Register between 1986 and 2012 by major diagnostic category. Adapted from Gibson et al.[59]



Nervous system — 4.1
Cardiovascular — 12.2
Respiratory — 1.8
Gastrointestinal — 6.4
Urogenital — 16.7
Musculoskeletal — 16.1
Chromosomal — 4.4
Metabolic — 1.4
Haematological/Immune — 0.5

■ Prevalence per 1,000 total births
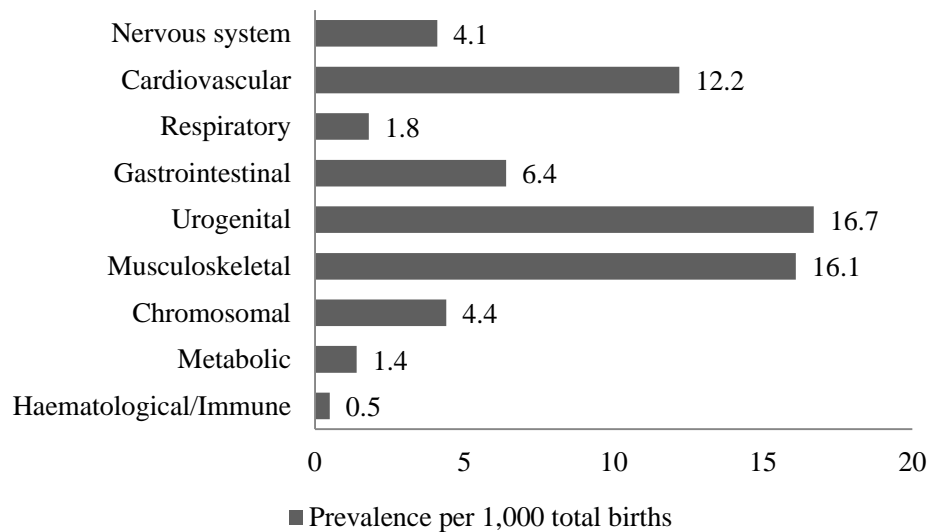
In 50% of cases the cause of the congenital anomaly is unknown. Genetic factors are estimated to be involved in 25% of cases, environmental factors in 10% of cases and 15% of cases are thought to be multifactorial (a combination of genes and the environment).[60] Genetic causes of congenital anomalies include chromosomal aberrations and single gene mutations. The most common chromosomal aberration is aneuploidy, that is, the loss or gain of a chromosome. Aneuploidy of autosomal chromosomes often leads to pregnancy loss.[60] An example of an autosomal aneuploidy seen in live born infants is Trisomy 21 or Down Syndrome. The prevalence of Down Syndrome in babies born in SA between 1986 and 2012 was 2.1 per 1,000 births.[59] One example of sex chromosome aneuploidy is Turner Syndrome (45, X karyotype), which had a prevalence of 0.3 per 1,000 births in SA over the same period.[59] Other rarer chromosomal abnormalities involve duplication, deletion or re-arrangement of part of a chromosome.[60]

Single gene mutations may occur on autosomal or sex chromosomes and may result in single or multiple anomalies. Cystic fibrosis is one example of a condition that is caused by an autosomal single gene mutation. This condition affected 1 in 2,500 SA births between 1986 and 2012.[59] An example of a disorder

that is caused by a single gene mutation involving sex chromosomes (X-linked disorders) is Duchenne Muscular Dystrophy, which affects about 1 in 3,600 – 1 in 6,000 males.[61]

Multifactorial congenital anomalies are caused by a combination of genetics and the environment. Neural tube defects, for example, show hereditary patterns in families; however the risk of these anomalies can be reduced by folic acid supplementation pre-pregnancy and in early pregnancy. Neural tube defects occurred in 1.6 per 1,000 births over the period 1986–2012.[59]

The fetus is particularly vulnerable to environmental toxins due to its fast rate of growth, the immaturity of its metabolic pathways, the presence of cells undergoing differentiation and vital organs in developmental stages.[62] Environmental causes of congenital anomalies include drugs/medications (e.g. alcohol), congenital infections (e.g. rubella), maternal disorders (e.g. diabetes mellitus) and physical agents (e.g. ionising radiation).[60]

Children conceived using ART have a greater risk of congenital anomalies compared to spontaneous conceptions. Meta-analysis of 45 cohort studies (92,671 ART infants) found that the risk of any congenital anomaly was 32% higher among ART infants (excluding OI and IUI) compared to non-ART infants. This increased to 36% when considering singletons births only and 42% when limited to major congenital anomalies. No increased risk of anomalies was found among multiple gestations, when analysis was limited to larger, high quality studies.[63]

It is unclear as to whether this observed increase is due to the fertility treatment or the diagnosis of infertility itself. Zhu et al.[64] found a significantly increased risk of congenital anomalies in the children of infertile couples (time to pregnancy greater than 12 months) who conceived naturally, hazard ratio 1.20 (95% CI 1.07–1.35) and in infertile couples who received treatment, hazard ratio 1.39 (95% CI 1.23–1.57) compared to fertile couples. Compared to fertile couples, an increased prevalence of nervous system, digestive system and musculoskeletal anomalies was observed among children of infertile couples (both untreated and treated).

When the analysis was limited to infertile couples, only an excess of genital organ abnormalities among children of treated infertile couples remained.[64]

In addition, Davies et al.[7] found that the type of fertility treatment received may alter the risk of congenital anomalies. In this study, there was no longer a significantly increased risk of anomalies among pregnancies conceived using IVF compared to pregnancies conceived without treatment after adjusting for parental factors. However, when the pregnancy was conceived using ICSI, the increased risk of anomalies remained significant. In relation to the treatment involving cryopreservation, a systematic review of 17 studies found no difference in the overall congenital anomaly rate between fresh and frozen embryos produced via IVF/ICSI,[65] with similar conclusions being drawn in a more recent review.[66]

**Rationale for investigating the fertility treatment population**

This section briefly outlines the key arguments in favour of investigating the fertility treatment population, including the growth of this population group, the increased rate of adverse events among this group and the availability of more extensive clinical data, which allows investigation of causal pathways for the association between infertility and adverse reproductive outcomes. Investigation of this population also provides an opportunity to unpack diagnoses among couples who are seeking treatment and to investigate the relative contribution of patient (infertility diagnosis, occupational exposures) and treatment factors to the outcomes of pregnancies conceived using fertility treatment.

*Growth of this population*

Since ART treatment began in Australia in 1979,[67] there has been a substantial increase in the number of treatment cycles initiated each year. In 1999, 26,579 cycles were undertaken in Australian and New Zealand compared to 73,598 in 2014.[30, 67] Figure 2 shows the growth in the number of ART cycles performed per 1,000 Australian women of reproductive age over the last 22 years.

There is substantial uptake of fertility treatments in Australia, with 50% of women who experience difficulties conceiving, seeking medical assistance.[15, 16] As a result of the increased uptake of ART by Australian couples, 4.4% of births in Australia are now the result of assisted conception.[30] In comparison, ART births in Europe (2008) ranged from 0.5% of total births in Turkey to 4.6% of total births in Denmark.[68] In the USA, this figure is 1.5% (2010).[69] Given the growth of ART in Australia, the ART population can now be considered a viable research population in its own right.

**Figure 2:** ART treatment cycles per 1,000 Australian females aged 15–44 years (1992–2014).
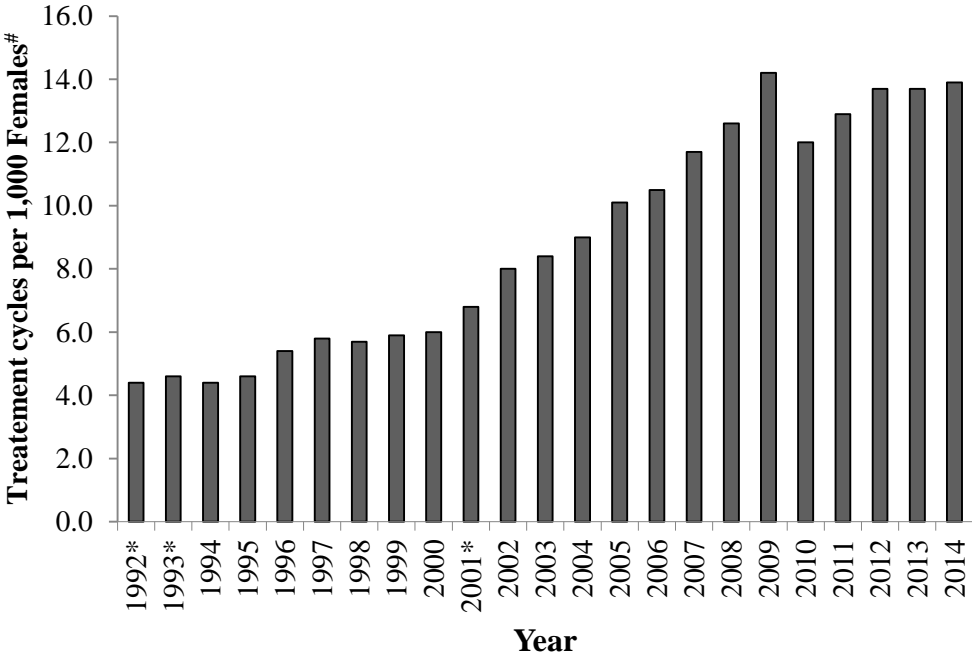


[#]Rates for 1992–2001 were estimated based on cycle data from the National Perinatal Epidemiology and Statistics Unit (NPESU) Assisted Reproductive Technology Reports and population data from the Australian Bureau of Statistics. Rates for 2002–2014 are taken directly from the NPESU reports.  *Combined Australian and New Zealand data.

*Increased occurrence of adverse effects and the causal pathway*

As described previously, adverse perinatal outcomes are more common among pregnancies from assisted conception than those where conception was natural. In

particular, increased prevalence of congenital anomalies, fetal death, preterm birth and low birth weight have been observed.[7, 70]

Variation in the occurrence of congenital anomalies by treatment modality may reflect differences in the invasiveness of treatment modalities, or the nature and severity of fertility problems in the parents.[71] Studies have found increased risk of congenital anomalies among subfertile couples who conceived spontaneously compared to fertile couples (with a shorter time to pregnancy).The increased risk of congenital anomalies associated with ART is diminished when analyses are adjusted for subfertility.[64, 72]

It is likely that subfertile couples who conceive naturally and those who conceive with treatment differ, not only in the severity of infertility, but also in terms of lifestyle and health behaviours.[73] Furthermore, it is possible that these patient factors create, not only a susceptibility to infertility, but susceptibility to adverse perinatal outcomes including congenital anomalies in offspring.[74, 75]

Overall, this suggests that subfertility and patient factors could play a role in the association between ART and congenital anomalies. Careful study design and analysis is required to separate these effects. Separation of the patient and treatment factors that contribute to adverse outcomes in ART births, which are now a substantial subgroup of the population, is also important for identifying potential strategies to prevent these outcomes from occurring.

## 2  Project One: Literature review on shift work and reproductive outcomes

**Objective**

This literature review aims to summarise the literature relating to the effects of night shift work on several reproductive outcomes including fertility and conception, pregnancy loss and selected perinatal outcomes including congenital anomalies, preterm birth and low birth weight. The following narrative review provides an overview of the existing evidence and indicates where there are gaps in the literature. A brief explanation of the potential mechanisms linking each outcome with circadian disruption is also provided. A subset of this review, including detailed critique of the literature, has been published and is presented at the end of project one.

**Methods**

Relevant epidemiological literature was located by conducting a search of the PubMed and Embase databases. The timeframe for the search in each database was January 1980 to February 2016 and only studies published in English were considered. The search terms used for each database are provided below.

*PubMed:*
(shift work[mh] OR shift work[tw] OR shiftwork[tw] OR night work[tw] OR night shift[tw]) AND ((infertility, female[mh] OR (female[tw] AND (infertil*[tw] OR subfertili*[tw] OR steril*[tw] OR fecundity[tw] OR time to pregnancy[tw]))) OR (abortion, spontaneous[mh] OR habitual abortion*[tw] OR spontaneous abortion*[tw] OR miscarr*[tw] OR pregnancy loss[tw] OR embryo loss*[tw] OR embryo death*[tw]) OR (stillbirth[mh] OR fetal death[tw]) OR (endometriosis[tw] OR menstrual[tw] OR ovulat*[tw] OR polycystic ovary syndrome[tw] OR PCOS[tw] OR (tubal[tw] OR fallopian[tw] OR idiopathic[tw] or unexplained[tw]) OR (assisted reproductive techn*[tw] OR reproductive

techniques, assisted[mh] OR embryo transfer*[tw] OR blastocyst transfer*[tw] OR fertilization in vitro[tw] OR in vitro fertili*[tw] OR fertilisation in vitro[tw] OR intracytoplasmic sperm injection*[tw] OR ICSI[tw] OR gamete intrafallopian transfer*[tw] OR oocyte donation*[tw] OR oocyte retrieval*[tw] OR oocyte aspiration*[tw] OR oocyte collection*[tw] OR oocyte pickup*[tw] OR ovulation induction*[tw] OR ovarian stimulation[tw] OR induced ovulation*[tw] OR sperm retrieval*[tw] OR sperm aspiration*[tw] OR zygote intrafallopian transfer*[tw] OR pronuclear stage transfer*[tw] OR fertility treatment[tw] OR infertility treatment[tw]))

*Embase:*

('shift work':de,ti,ab OR shiftwork:ti,ab OR 'night work':ti,ab OR 'night shift':ti,ab) AND ( ('spontaneous abortion':de,ti,ab OR 'habitual abortion':ti,ab OR miscarriage:ti,ab OR 'pregnancy loss':ti,ab OR 'embryo loss':ti,ab OR 'embryo death':ti,ab) OR (stillbirth:de,ti,ab OR 'fetal death':ti,ab OR 'perinatal death':ti,ab OR 'neonatal death':ti,ab OR 'perinatal mortality':ti,ab) OR ('female':ti,ab AND (subfertility:ti,ab OR sterility:ti,ab OR infertility:ti,ab OR fecundity:ti,ab)) OR endometriosis:de,ti,ab OR menstrua:ti,ab OR ovulat:ti,ab OR 'polycystic ovary syndrome':de,ti,ab OR PCOS:ti,ab OR tubal:ti,ab OR fallopian:ti,ab OR idiopathic:ti,ab or unexplained:ti,ab OR ('assisted reproductive technology':ti,ab OR 'embryo transfer':ti,ab OR 'blastocyst transfer':ti,ab OR 'fertilization in vitro':de,ti,ab OR 'intracytoplasmic sperm injection':ti,ab OR 'gamete intrafallopian transfer':ti,ab OR 'oocyte donation':ti,ab OR 'oocyte aspiration':ti,ab OR 'oocyte collection':ti,ab OR 'oocyte retrieval':ti,ab OR 'ovulation induction':ti,ab OR 'ovarian stimulation':ti,ab OR 'sperm retrieval':ti,ab OR 'sperm aspiration':ti,ab OR 'zygote intrafallopian transfer':ti,ab OR 'pronuclear stage transfer':ti,ab OR 'fertility treatment':ti,ab OR 'infertility treatment':ti,ab))

Cross-sectional, case-control and cohort study designs were included as well as systematic reviews and meta-analyses. Studies were excluded if there was no control group. For some outcomes, there was a sizable existing literature. Therefore where available, this review focusses on the findings of systematic reviews and meta-analyses, supplemented by evidence from individual studies published subsequently. Where possible, the type of shift work considered in each

reviewed study is specified. However, despite night and rotating shift work having the greatest impact on biological and social functioning, not all studies are able to distinguish between different types of shift work, and thus report on exposure to 'shift work' in a broad sense.

The review is structured as follows. Firstly the definition and epidemiology of shift work are provided, followed by a description of the link between shift work and circadian disruption. The review then summarises the literature relating to the association between shift work and female subfertility (including time to pregnancy, reproductive conditions, use of fertility treatment), pregnancy loss (miscarriage and fetal death) and perinatal outcomes (preterm birth, small for gestational age, low birthweight and congenital anomalies). The review concludes with a discussion of the difficulties associated with epidemiological study of shift work and reproductive outcomes, and a summary of gaps identified in the literature.

## 2.1  Literature review

**What is shift work?**

Increasing demand for 24-hour access to goods and services necessitates that workers depart from the 'nine to five' work schedule. Pressure to accept more flexible working conditions means that more people are engaged in shift work that allows activity to occur around the clock. While this satisfies employer and societal demands, it creates potential health and social problems for the workers involved. Some occupations such as nursing have always entailed working overnight; however growth in other related sectors including aged care means that demand for night shift workers is increasing. This has significant implications for women, as although both men and women are employed in this sector, female workers predominate.[2]

Shift work is defined as the organisation of working hours whereby different individuals or teams work in succession. This allows work to continue up to 24 hours a day.[76] Shift work can be further defined by the type and schedule of work.

Types of shifts include morning, afternoon, evening, and night shifts and the schedule of shifts performed by the worker may be, either the same shift all the time (permanent) or rotating in a clockwise/anticlockwise fashion.[77]

In Australia, 1.5 million (16%) of those in paid employment are involved in shift work.[1] Similar prevalence of shift work is found in other developed countries. For example, the average prevalence of shift work among workers in the European Union in 2010 was 17%.[78] In the USA, approximately 17.7% of workers aged over 16 years work non-daytime shifts.[79]

Among Australian women engaged in shift work, the majority work rotating shifts (41%). The highest proportion of female shift workers is found in the 15–19 year age group (22.3%) and 67% of all shift working women are aged less than 45 years. Shift work is most common in the Accommodation and Food Services (33%) and Health Care and Social Assistance (30%) industries. Occupations with the highest proportions of shift workers include 'Machinery operators and drivers' (31.0%) and 'Community and personal services workers' (28.5%).[1]

**Shift work and circadian disruption**

Disruption of circadian rhythms is a key mechanism through which night and rotating shift work produce ill health.[4] The circadian rhythm is the 24-hour biological cycle that regulates sleep and wakefulness in humans. It is coordinated by the suprachiasmatic nucleus (SCN) in the hypothalamus and is synchronised with environmental stimuli. [4, 80] While exposure to light/dark is the strongest determinant of circadian entrainment, other factors including temperature, activity and food intake are also involved.[81] The SCN relays circadian information to other central circadian oscillators such as the pituitary and pineal glands, and peripheral oscillators such as the thyroid gland and gonads, via the regulation of clock-gene expression and neuroendocrine signalling.[82, 83]

There are two main pathways through which the SCN relays circadian signals throughout the body. The first occurs via the central and peripheral nervous system; the second is via the rhythmic production of melatonin by the pineal

gland.[80] Melatonin secretion is normally higher at night time, but when exposure to light at night occurs, this secretion can be reduced or altered in timing.[84]

Disruption of circadian signals distributed by the nervous system or melatonin can result in phase shift. Phase shift occurs when peripheral biological activities, such as digestion, become asynchronous with the central sleep/wake cycle. Phase shift also affects metabolic and hormonal activity, which may contribute to long term health effects such as obesity and impaired insulin metabolism,[85] as well as mood and cognitive disorders.[80]

Physiological adaptation to phase shift varies among individuals and for different hormones. A lack of adaption of melatonin secretion to a night shift schedule has been reported, although the prevalence of adaption is controversial and may relate to the type of shift work schedule. For example, a review of six studies involving permanent night shift workers (combined n = 76) found that ≤3% achieve complete entrainment, ≤25% show partial entrainment and ≥72% showed no adaptation.[86] Conversely, a small study (n = 15) of police officers who usually worked a rotating schedule found that 44% were adapted to the night schedule in terms of melatonin secretion patterns. Adaptation of cortisol rhythms is also variable. A study of health care night shift workers, cortisol secretion took five consecutive night shifts to adapt to the new sleep/wake pattern, and 25% of participants showed no adaptation at all.[87]

It is therefore, important to appreciate that adaptability and tolerance to night work varies between individuals.[88] Those who work permanent night shift for long periods are likely to be self-selected for this schedule due to high tolerance of this regimen. Some individuals may cease night work due to impacts on health, or because they feel they cannot cope or function effectively under this schedule. Factors that influence both physiological and psychological tolerance include light exposure during and after night shifts, whether sleep can be managed and achieved in time off work, chronotype (being a 'morning' or 'evening' type), age, general health and fitness.[89, 90] Personal circumstances may also be relevant, such as family responsibilities which can affect ability to make up sleep.[91]

Furthermore, the nature of the work can affect tolerance, particularly the degree of control a worker has over their work schedule.[92]

Much of the evidence linking circadian disruption and reproductive biology comes from work in animal models and is yet to be demonstrated unequivocally in humans. Nevertheless, there appears to be potential for circadian disruption to impact successful reproduction through misalignment of reproductive hormone secretion and function due to incomplete adaptation. Melatonin hormone has been found in reproductive tissues including the ovary and placenta, where it appears to play an important role in protecting these tissues from reactive oxygen species.[80] Furthermore, circadian clock-gene expression has been observed in several reproductive tissues including the ovary, uterus and placenta, and melatonin receptors are found in organs of the reproductive system.[93]

**Shift work and female subfertility**

*Time to pregnancy*

Fertility among shift workers has been investigated by measuring time to pregnancy (in months) compared to non-shift workers. One systematic review with meta-analysis that considered time to pregnancy and one subsequent study were identified. Stocker et al.[94] performed a meta-analysis of data from five study cohorts investigating the risk of infertility (defined as time to pregnancy of >12 months) among shift workers compared to non-shift workers. Although the unadjusted OR suggested significantly higher risk of infertility among shift workers (OR = 1.80, 95% CI 1.01–3.19), this was not the case after consideration of potential confounders (OR = 1.12, 95% CI 0.86–1.44). Only one of the included studies considered fixed night shift workers, again finding that elevated risk of infertility among night shift workers could be explained by confounding factors such as age, gravidity, lifestyle and occupational factors. There was limited consideration of male factors that may influence time to pregnancy in the included studies. All considered the male partner's occupation and one considered male smoking.

Time to pregnancy (in months) was also examined in the Nurses' Health Study 3.[95] In this study, data were obtained from 1,739 employed nurses who were attempting to become pregnant. After taking into account a number of potential confounders, there was no difference in time to pregnancy for any of the shift work schedules (evening only, night only, rotating shifts with or without nights) in comparison to fixed daytime work. Characteristics of the male partner were not available in this study.

*Menstrual irregularity*

Altered menstrual function in female shift workers may be one reason why higher rates of subfertility are observed in this group. A meta-analysis of six cohorts from studies of shift work and early reproductive outcomes found that female shift workers were significantly more likely to experience menstrual irregularity (defined as cycle length of <25 or >31 days) after adjusting for potential confounders (OR = 1.15, 95% CI 1.01–1.31). The effect was even stronger when the analysis was limited to nightshift workers versus non-shift workers (OR = 1.72). However this result did not reach statistical significance due to small sample size in this sub-group.[94]

A more recent study published after the systematic review by Stocker et al. investigated menstrual disturbance among more than 6,000 nurses participating in the Nurses' Health Study 3.[96] The menstrual cycle characteristics of nurses working specific shift schedules including evening only, night only, rotating shifts with nights and rotating shifts without nights were compared a reference group consisting of day time workers. Working nights only or rotating shifts with nights was associated with increased likelihood of irregular cycles (OR = 1.32, 95% CI 1.15–1.51 and OR = 1.27, 95% CI 1.10–1.47, respectively). The frequency of night shifts per month was also associated with menstrual irregularity. There was some indication that women whose schedule involved rotating shifts with nights were more likely to experience short (OR = 1.75, 95% CI 0.98–3.12) and long (OR = 1.28, 95% CI 1.03–1.61) cycle lengths compared to fixed day workers. Cycle length was not associated with any other shift type. Evening shift and

rotating shift work without nights were not associated with irregularity or cycle length. All results were adjusted for potential confounders (including smoking, body mass index, and physical activity).[96]

Different hormone systems follow different secretory patterns and adapt at different rates to circadian disruption.[82, 97] Misalignment of cortisol and reproductive hormones, as well as the interaction of melatonin with these hormonal rhythms could be an underlying mechanism for impaired menstrual function and fertility in shift workers.[82, 98, 99] Animal studies suggest that optimal functioning of the SCN is required to produce the LH surge and ensuing ovulation and that melatonin interacts with gonadotropins, including augmentation of the LH surge.[82, 100] Evidence from rats and humans also suggests the timing of the LH surge is strongly correlated with that of the diurnal peak in cortisol.[97] Longer cycles are associated with a longer follicular phase and delayed ovulation.[101] Augmentation of the timing of the LH surge may prolong the follicular phase of the menstrual cycle and delay ovulation. This shifting of the time of ovulation may contribute to increased time to pregnancy.

*Endometriosis*

As previously described, endometriosis is a chronic oestrogen-dependent, inflammatory disorder that is characterised by the presence of endometrial glands and stroma outside of the uterine cavity.[25] An association between shift work and self-reported endometriosis was first identified in a small Norwegian case-control study.[102] This study found a significantly higher prevalence of shift work among cases (OR = 1.8, 95% CI 1.1–3.0). This study was unable to adjust for potential confounders and concluded that the association with shift work was due to nulliparity among women with endometriosis.

A more recent population-based case-control study found an increase in the risk of laparoscopically-confirmed endometriosis among night shift workers, especially those who worked night shift more than 50% of the time (OR = 1.98, 95% CI 1.01–3.85), compared to day shift workers.[103] This result remained after adjustment for parity. The risk of disease was further elevated among women who

changed their sleeping patterns on days off and those who had longer durations of night shift work.

Using data from the same study, Marino et al.[104] went on to investigate specific occupations in which the risk of endometriosis was increased. Women who worked as flight attendants, service station attendants and healthcare workers (particularly nurses and nursing aides) had higher risk of endometriosis. These results were independent of income and education levels. Although, statistically significant, the wide confidence intervals for these results suggest that the estimate of effect was imprecise owing to small numbers. These associations warrant further investigation in higher powered studies, and study designs that avoid potential recall bias from the self-reporting of exposure information by cases and controls.

A prospective study of women participating in the Nurses' Health Study II found higher rates of self-reported laparoscopically-confirmed endometriosis (OR = 1.71, 95% CI 1.18–2.49) among rotating night shift workers (working at least three nights per month) compared to fixed day workers, but only among those who had done shift work for at least five years and who concurrently reported infertility (time to pregnancy >12 months).[105] This suggests that some individuals may be more susceptible to the effects of circadian disruption on reproductive health, or that circadian disruption exacerbates the severity of endometriosis, leading to impaired fertility. Alternatively, nurses who wanted to become pregnant may have been more likely to undergo investigations for potential infertility factors, such as endometriosis verified by laparoscopy, compared to nurses who did not want to become pregnant and thus did not report infertility.

Hormonal disturbances, along with immunological and inflammatory pathways, are proposed mechanisms linking shift work with endometriosis in susceptible individuals.[105] Exposure to light at night during night shift work can reduce melatonin hormone secretion.[106] Melatonin exhibits an antagonistic relationship with estradiol and has been shown to inhibit aromatase activity.[107] As endometriosis is an estrogen-dependent condition, altered estrogen metabolism in

night shift workers may increase their susceptibility to endometriosis, but this has yet to be confirmed in clinical studies.[105, 108]

Neuroendocrine stress (increased cortisol and catecholamine activity), oxidative stress, altered immune function and low-grade system inflammation are also induced by circadian misalignment and poor sleep.[109] Impaired immune surveillance and reactive oxygen species have been implicated in the inflammatory and pathophysiological processes involved in the development of endometriosis.[108, 110, 111] Therefore, impaired immune function and inflammatory responses in shift workers may contribute to increased susceptibility to this disease.

Although these mechanism have been proposed, it should be noted that the exact cause of endometriosis is not well established. One theory is that the condition is caused by retrograde flow of menstrual debris outside of the fallopian tubes.[112] Depending on when and how endometriosis is diagnosed in studies of shift working women, it is possible that what is being detected is an exacerbation of pre-existing endometriosis due to hormonal, immune or inflammatory disturbances, rather than new onset endometriosis. As mild endometriosis may be asymptomatic and definitive diagnosis requires laparoscopy,[113] establishing whether shift work is associated with new onset endometriosis would be very difficult, even in prospective studies.

*Polycystic ovary syndrome*

One study investigating the association between shift work and the reproductive and metabolic symptoms that are characteristic of PCOS was identified. This cross-sectional study of 231 women did not focus on the outcome of PCOS specifically, but the presence of abnormalities that are typical of this condition in healthy volunteers. Shift work was not significantly associated with any of the ovarian or androgenic parameters that are characteristic of PCOS. Waist to hip ratio was slightly, but significantly, elevated among shift workers, but there were no differences in other metabolic parameters (such as blood pressure, cholesterol, insulin resistance) in shift workers compared to non-shift workers.[114]

*Other female factor fertility diagnoses*

No studies investigating the prevalence of tubal factor infertility or idiopathic (unexplained) female factor infertility among shift workers could be located.

**Shift work and use of fertility treatment for conception**

No studies investigating the use of fertility treatment for conception among shift workers could be located.

One previous study considered the association between women's occupational stress and ART conception and delivery rates using a small cohort (n = 75) of women attending fertility clinics for female factor infertility.[115] Involvement in shift work was used as a measure of workload, however conclusion could not be drawn about the effect of shift work on treatment outcomes as only nine women reported any shift work.

**Shift work and pregnancy loss**

*Miscarriage*

Clinically, miscarriage is defined as the delivery of an embryo or fetus before 20 weeks' gestation.[9] However, as evident below, many epidemiological studies define miscarriage as a pregnancy loss at <25 or <28 weeks gestation. This definition takes into account the gestational age at which a fetus is considered viable, separating miscarriage from very preterm delivery. If delivered between 25 and 28 weeks, most fetuses are considered to be viable, with advanced care.

There have been several reviews on the topic of shift work and pregnancy loss, including systematic reviews with meta-analysis (Table 1).[5, 94, 116] Although there is some overlap in the included studies for each meta-analysis, the general consensus shows an elevated risk of miscarriages among female shift workers, particular among pregnancies to women engaged in fixed night work. Common

themes that arose when interpreting the quality of studies considered in these review included a lack of prospective studies, self-reported outcome or exposure data that could be open to bias, variation in the definitions of both shift work and miscarriage. There were also differences in the extent to which included studies could adjust for potential confounders, although most adjusted for at least some.

A subsequent study investigated women's work schedule and miscarriage (loss before 22 weeks of gestation) using data on 88,373 pregnancies contained in the Danish National Birth Cohort.[117] Several work schedules were considered: fixed day shift (reference), fixed evening shift, fixed night shift, rotating shifts without nights, rotating shifts with nights, and not working outside the home. The risk of miscarriage was significantly higher among women who worked rotating shifts with nights (OR = 1.21, 95% CI 1.06–1.39) compared to fixed day workers. The risk for fixed night shift workers was elevated but not statistically significant (OR = 1.25, 95% CI 0.89–1.82), perhaps reflecting the relatively small sample size (n = 670 pregnancies) for this analysis. No other shift work schedules were found to be associated with miscarriage. These results were adjusted for several potential confounders including maternal age, parity, pre-pregnancy weight, exercise, smoking, alcohol and coffee consumption during pregnancy, and household occupational status.

A potential mechanism underpinning miscarriage (and possibly delayed conception) among female shift workers may relate to oocyte quality. Melatonin is a potent antioxidant.[118] It is hypothesised that melatonin is involved in oocyte development and may play an important role in protecting the oocyte from reactive oxygen species produced during ovulation, hence reducing the risk of DNA damage.[80, 110] This is further supported by the observation of improved oocyte quality and increased fertilisation rates among IVF patients who were treated with melatonin.[110, 119]

**Table 1:** Summary of systematic reviews investigating the association between shift work and miscarriage.

| Study | Population | Definition of shift work | Definition of miscarriage | Studies included | Summary finding |
|---|---|---|---|---|---|
| Quansah et al. 2012 | Female nurses only (Total n = 11,616) | Unclear how different types of shift work reported in included studies were combined for meta-analysis. | Varied: loss at <29 weeks, loss at <20 weeks, ICD8 classification. | 4 studies published between January 1966 – August 2009 | OR = 1.44 (1.06–1.95) |
| Bonde et al. 2013 | General population, specific occupational groups | Meta-analysis considered: 1) Fixed night work vs day work (total n = 44,756). 2) Rotating 3-shift schedules or evening/night shifts vs day or 2-shift schedules (total n = 50,708). | Loss between clinical recognition of pregnancy and 20–28 weeks. | 12 studies published between 1966 – June 2012 | Fixed night vs day work RR = 1.51 (1.27–1.78) Night or rotating 3-shift vs day or 2-shift RR = 1.12 (0.96–1.30) |
| Stocker et al. 2014 | General population, specific occupational groups | Shift work defined as work outside of 8:00 AM to 6:00 PM (Total n = 23,604). Subgroup analysis of women who only worked night shift vs non-shift workers (total n = 13,018). | Early spontaneous loss before 25 weeks. | 7 studies published up to July 2013. 5 out of 7 studies included in subgroup analysis. | Shift work vs non-shift work: Adjusted OR = 1.04 (0.89–1.22). Night shift vs non-shift work: Adjusted OR = 1.41 (1.22–1.63). |

*Fetal death*

The World Health Organisation defines fetal death or stillbirth as spontaneous pregnancy loss after 20 weeks gestation.[9] Three studies could be located that considered late pregnancy loss among shift workers, although none strictly applied this definition.

Two studies conducted in Japan and Sweden in the 1980's considered fetal deaths among women who worked shift work.[120, 121] In both studies, the number of fetal deaths was small, therefore making comparisons between groups difficult. The Japanese study reported three fetal deaths (not further defined) out of 128 pregnancies to shift workers, compared to no fetal deaths among 101 pregnancies to day workers.[121] The Swedish study considered fetal death (defined as loss after 27 weeks gestation and without congenital anomalies) among 18,511 singleton pregnancies and observed four fetal deaths among women who reported a changing shift roster. This was not significantly more than expected among all working women (adjusted O/E ratio = 0.61, p > 0.1).

A later study by Zhu et al.[122] reported increased risk of fetal death (loss after 28 weeks) among fixed night shift workers (n = 420 pregnancies) compared to day workers (n = 33,694). The hazard ratio (HR) was elevated but not statistically significant, HR = 1.92 (0.59–6.24). Other types of shift work were also considered, including rotating shift work with nights, but there was no difference in the risk of fetal death compared to day workers.

The results of these studies (and indeed the lack of evidence for this outcome in general) are likely to partly reflect the rarity of fetal death as an outcome. Also, the overall prevalence of fixed night shift is also relatively low. Zhu et al.[122] suggest that previous studies on the topic of shift work and pregnancy loss lack statistical power due to small sample sizes and were potentially biased by retrospective data collection and ascertainment of outcome data via maternal recall. Their study overcame issues relating to bias through its prospective design and use of registry data; however, it could not overcome issues associated with power.

The major risk factors for fetal death in developed countries include maternal obesity and smoking, advanced maternal age, condition such as diabetes and hypertension and pregnancy complications such as fetal growth restriction.[123] Several of these factors occur at higher rates among shift workers, especially those related to lifestyle and metabolic health.[124] The above studies were able to consider some of these potential confounders (excluding fetal growth restriction, which is likely to sit on the causal pathway), but none considered maternal conditions in pregnancy.

**Shift work and perinatal outcomes**

*Preterm birth, small for gestational age, low birth weight*

A meta-analysis of 19 studies published between 1966–2011 found a small (OR = 1.14, 95% CI 1.01–1.30), but statistically significant, increase in the risk of preterm delivery among pregnant shift workers.[125] When this was limited to 12 higher quality studies, the risk estimate was reduced and no longer statistically significant (OR = 1.04, 95% CI 0.95–1.15). Shift work was defined as shift or night work.

Eight studies included in this meta-analysis considered shift work exposure after the first trimester, although only three included the third trimester.[125] The pooled OR for exposure later in pregnancy was 1.17 (95% CI 0.86–1.60), suggesting little difference in risk when exposure continues later in to pregnancy.

One other study has been published since this meta-analysis. This population-based prospective cohort study of 4,680 pregnant women found no association between occasional night shift work and preterm birth. There was a higher risk of preterm birth among women who reporting working night shift often (OR = 1.29, 95% CI 0.46–3.65), but this was not statistically significant.[126] Results were adjusted for potential confounders including maternal age, height, weight, education level, ethnicity, parity, smoking, alcohol used, folic acid supplementation, self-perceived health and baby sex.

Van Melick et al.[127] later conducted a meta-analysis, which included the above cohort study by Snijder et al.[126], in addition to 10 studies of high-moderate quality from the earlier review by Palmer et al.[125] The overall pooled results remained unchanged with the inclusion of this additional study (OR = 1.04, 95% CI 0.90–1.20).

Small for gestational age (SGA) was also considered in the meta-analysis described above. Again, results suggested that there is not a strong association between shift work and SGA offspring. The pooled OR for 10 studies was 1.01 (95% CI 0.92–1.10). There was little difference in result when the analysis was restricted to seven higher quality studies or five studies th at considered exposure later in pregnancy.[125] The recently published study by Snijder et al. also considered SGA. A non-significant increase in risk of SGA was found when women occasionally worked night shifts during pregnancy (OR = 1.69, 95% CI 0.86–3.33) and a non-significant decrease was observed among those working night shifts often (OR = 0.73, 95% CI 0.17–3.08). The width of the confidence interval for the 'often' groups reflects the small number of women in the study who often worked night shift (n = 60).

Investigation of low birth weight (LBW) among offspring of shift workers shows slightly higher effect estimates, however, as with the other fetal growth outcomes described above, the estimates are imprecise. Palmer et al.[125] suggest that this reflects the reduced number and quality of studies that investigate this outcomes. A pooled analysis of low birth weight (either as a continuous or categorical variable) was not performed in the most recent meta-analysis describe above, however they did report a median relative risk of 1.28.[125] An earlier meta-analysis of six studies by the same lead authors produced an odds ratio of 1.27 (95% CI 0.93–1.74). Snijder et al. showed increased risk of low birth weight among pregnant women who occasionally (OR = 1.78, 95% CI 0.64–4.96) and often (OR = 1.23, 95% CI 0.28–5.41) worked night shifts, but again neither result reached statistical significance.[126]

Overall, the epidemiological evidence points to a slight effect of maternal shift work on preterm delivery and measures of fetal growth. Despite this, potential mechanistic pathways have been identified, mainly through animal and in vitro studies. Maternal circulating melatonin establishes the circadian rhythm of the fetus,[128] however there is also evidence that the placenta is capable of producing its own melatonin.[129] This placental melatonin is secreted in a non-rhythmic fashion and is likely to promote optimal placental function by regulating apoptosis and protecting from oxidative stress and cellular degeneration.[93, 129, 130] Melatonin has also been shown to stimulate the production of hCG in the placental trophoblasts.[131] This provides another potential mechanism for adverse perinatal outcomes whereby shift-work-induced deficiencies in overall melatonin lead to lower hCG production and placental insufficiency.[93]

### *Congenital anomalies*

Only two previous studies investigating maternal shift work and congenital anomalies could be found. A small study of 160 time-matched case-control pairs found an elevated risk (OR = 1.5) of cardiovascular anomalies among children of shift working mothers. This result was not statistically significant.[132] It is not clear how shift work was defined in this study and, as a consequence of the sample size, few women were exposed to occupational hazards. A larger study (1,475 case-control pairs) found no association (all ORs close to unity) between maternal shift work and pooled or specific anomalies.[133] Urogenital anomalies were not assessed. Shift work was defined as two-shift, three-shift or other shift work, however the extent to which study participants were involved night work was not apparent.

Despite a lack of epidemiological evidence for an association between shift work and congenital anomalies, there are plausible mechanisms through which circadian disruption could be teratogenic. Endocrine disruption and oxidative stress are two mechanisms that are implicated in the development of congenital anomalies.[134] Hormonal changes produced by circadian disruption therefore provide a potential mechanism for the development of congenital anomalies in the children of female shift workers. This is particularly relevant to the development

of the urogenital tract in male offspring, which is dependent on androgen-estrogen balance. There is some evidence of elevated sex hormone secretion among premenopausal shift workers and women exposed to light at night. Elevated estradiol levels have been found among pre-menopausal rotating shift workers,[135] although this has not been found in other studies. Another study found higher levels of FSH and LH, but not estradiol among night shift nurses compared to day shift nurses.[106] When analysis was conducted within the night shift group, significantly higher estradiol levels were found during daytime sleep and night-time work, compared to night-time sleep. The authors suggest that the effect of shift work on estrogen is small, or that variability in estrogen levels is too great to detect differences between the day and night shift workers. Duration of exposure to shift work also appears to influence the association with sex hormone levels.[135]

As described above, melatonin plays an important role in maintaining placental functioning and protecting the developing fetus from oxidative stress. Build-up of reactive oxygen species can produce irreversible damage to cellular macromolecules including DNA, proteins and lipids, and can also alter gene expression. As well as impairing fetal growth, oxidative damage has been linked to several classes of anomalies including musculoskeletal, neural and cardiac anomalies.[134]

**Difficulties in studying shift work and reproductive outcomes**

As alluded to above, there are a number of issues that make it difficult to conduct epidemiological studies of shift workers. These are described in more detail below.

*Definition of shift work and types of shift work*

The definitions of shift work vary across studies and jurisdictions, with many studies combining all types of shift work into a single variable. While this approach may improve the power of a study, it does not take into account evidence that rotating shift work and night shift work are more likely to produce circadian disruption and phase shift.[136]

The definition of night shift also varies from country to country. In Australia, an evening, night or graveyard shift involves work during the hours of 5.00 pm to 6.00 am.[1] In the USA, night shift is any shift that starts between 10.00 pm – 2.00 am and ends between 5.00 am and 8.00 am.[137] The definition of night shift varies across countries in Europe, but generally refers to any work during the normal hours of sleep, but specifically including the period between 12.00 am – 5.00 am.[136, 138]

Schedules of shift work also vary widely and this makes it difficult to consider all possibilities in a study. For example, whether shift work is regular or irregular, the direction of rotating shifts (forward or backward rotating), the duration of each cycle of rotating shifts (e.g. number of day shifts, number of evening and number of night shifts) and the number of years that a woman has been engaged in shift work, could impact the extent to which shift work influences health.

### *Potential confounders and unmeasured factors*

When interpreting the evidence regarding shift work and reproductive outcomes, it is important to consider the role of confounding lifestyle factors and health behaviours that are common among shift workers. These include smoking, obesity, poor diet and inadequate exercise.[139] Many of the above reproductive and perinatal outcomes are influenced by maternal characteristics, particularly obesity, smoking and metabolic conditions such as hypertension and diabetes.

Sleeping patterns and disturbances are also important. Sleeping patterns of women when working night shift and on days off can affect their vulnerability to reproductive disorders, such as endometriosis and menstrual irregularity.[103, 140] This is related to tolerance, discussed previously.

Apart from menstrual irregularity and endometriosis, the outcomes described above are influenced by characteristics of not only the female, but also the male and the couple. Therefore, it may be important to consider such characteristics

during data collection and analysis. In studies of female shift workers, the ability to consider male factors varies greatly between studies.

Finally, for perinatal outcomes such as preterm birth, the timing of exposure needs to be considered. It is important to determine whether women were working night shifts during the third trimester of pregnancy and when they ceased working. If women who would normally work night shifts alter their shift schedules or cease work earlier during pregnancy, this may reduce the likelihood of observing an adverse outcome and may explain why there is little evidence of preterm birth among female shift workers. As seen in a prospective cohort study conducted by Bollati et al.[141] 130 women (10% of the cohort) were working night shift at 11 weeks gestations, compared to 33 (4%) at 34 weeks.

### *Problems with sample size*

Many of the studies described above found elevated risk of adverse outcomes among shift workers that were not statistically significant. In some cases, this could truly mean that there is no association, in some cases it might be a consequence of insufficient sample size. Thus p-values greater than 0.05 cannot be treated as reassuring. Exceptionally large odds ratios and/or upper confidence limits are, in some cases, signs of data sparsity and insufficient power,[142] which is most likely to occur in studies of rare exposures (such as fixed night shift) or rare outcomes (such as congenital anomalies and fetal death).

Participant recruitment in studies that measure hormone levels in blood or urine may be limited by the invasiveness and cost of obtaining and processing biological samples. This is an important limitation for studying the effects of shift work as there are large inter-individual variations in melatonin secretion among night shift workers.[98, 99] This is also likely to be the case for estrogen.[106]

## Other biases resulting from sample selection processes and study design

There are several examples of how study design and the selection of study participants can introduce bias in studies of shift work and reproductive outcomes. The first applies to any study of fecundability and time to pregnancy, rather than the study of shift workers. In most examples, studies of fecundability and time to pregnancy are conducted retrospectively among couples who did manage to conceive a pregnancy. This excludes couples with more severe subfertility who do not conceive spontaneously. This can also apply to prospective studies, where follow up time is insufficient to capture time to pregnancy in subfertile couples. A second caveat with this approach is that pregnancies must be planned, which is not often the case.[143]

With any study of shift workers, it is important to consider that individuals with greater physiological tolerance and/or more adaptable psychosocial conditions may self-select into specific shift work patterns.[144] Either of which may induce a form of selection bias, or 'reverse causation'.

In any study of reproduction in occupational cohorts, there is the chance that an 'infertile worker effect' is occurring.[143] For example, women who fall pregnant quickly or who do not suffer miscarriages are less likely to be in the workforce and are therefore less likely to be selected for study participation. This may inflate the prevalence of subfertility and miscarriage among working women. Further, shift workers who experience work-family conflict are more likely to leave there job.[145] This could lead to an "infertile shift worker effect" where cohorts of shift workers appear more subfertile due to women with children self-selecting out of shift work in favour of more family friendly schedules.

**Summary of gaps in the literature**

This review has identified several key gaps in the literature that warrant further investigation. Firstly, aside from menstrual irregularity and endometriosis, there has been very little investigation of other clinical infertility diagnoses among female shift workers. It is unknown whether shift work and associated circadian misalignment contributes to other reproductive conditions such as ovulatory infertility. Secondly, the requirement for, and uptake of fertility treatment by shift working women, and the outcomes of fertility treatments among these women has not previously been investigated.

Finally, while perinatal outcomes such as preterm birth and low birth weight have been considered by several studies including meta-analyses, there has been limited consideration of congenital anomalies among children born to shift workers. This is particularly true in relation to urogenital anomalies.

## 2.2 Introduction to published review

The following manuscript is the key output from project one of this thesis. It represents a multidisciplinary piece of work that summarises a complex literature relating to night shift work, reproductive health and fertility (including time to pregnancy, menstrual irregularity and endometriosis) and miscarriage. This review goes beyond collating the evidence to include a critical analysis of the epidemiological literature. For each outcome under consideration, it provides an assessment of the overall state of the evidence, and prudent responses in line with public health principles. The manuscript was published in 2016 in a special issue of Seminars in Reproductive Medicine entitled 'Lifestyle in Reproductive Medicine'. The content, style and format of this manuscript were developed with the journal's target audience in mind, that is, those involved in clinical pre-conception and early pregnancy care. In line with the issue theme and target audience, an important consideration was to provide policy directions and practical options that could be used to alleviate the effects of night shift work on reproductive health.

For this manuscript, I formally assembled a group of academics whose expertise spanned basic biology to population-based epidemiology, including an international co-author, Professor Scott Davis, who is an expert in chronobiology and was a member of the International Agency for Research on Cancer Working Group that classified shift work as a probable human carcinogen.[136] Other co-authors provided expertise in reproductive epidemiology (Dr Jennifer Marino, Dr Melissa Whitrow, and Professor Michael Davies), circadian biology (Dr Tamara Varcoe), lifestyle factors and infertility (Dr Lisa Moran), perinatal epidemiology (Dr Alice Rumbold), reproductive biology (Dr Hannah Brown) and social epidemiology (Professor Vivienne Moore).

My contributions to the manuscript as first author were as follows:
- Contributed to development of the manuscript topic and structure.
- Conducted the main epidemiological literature searches.

- Identified experts in appropriate fields and co-ordinated their contributions as co-authors.

- Drafted the 'Introduction', 'Defining and Quantifying Shift Work', 'Review of the epidemiological literature' and 'Conclusion' sections of the manuscript.

- Critically reviewed all contributions and requested clarification or additional material form co-authors.

- Compiled the complete review, responded to assessors' reports and made revisions, and acted as corresponding author.

## 2.3 Statement of authorship

# Statement of Authorship

| Title of Paper | Fixed or rotating night shift work undertaken by women: implications for fertility and miscarriage. |
|---|---|

| Publication Status | ☑ Published      ☐ Accepted for Publication |
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| | ☐ Submitted for Publication    ☐ Unpublished and Unsubmitted work written in manuscript style |

| Publication Details | Fernandez RC, Marino JL, Varcoe TJ, Davis S, Moran LJ, Rumbold AR, et al. Fixed or rotating night shift work undertaken by women: implications for fertility and miscarriage. Semin Reprod Med. 2016;34(2):74-82. |
|---|---|

## Principal Author

| Name of Principal Author (Candidate) | Renae Clare Fernandez |
|---|---|
| Contribution to the Paper | Contributed to development of the manuscript topic and structure. Conducted the main epidemiological literature searches. Identified experts in appropriate fields and coordinated their contributions as co-authors. Drafted the 'Introduction', 'Defining and Quantifying Shift Work', 'Review of the epidemiological literature' and 'Conclusion' sections of the manuscript. Critically reviewed all contributions and requested clarification or additional material form co-authors. Compiled the complete review and acted as corresponding author. |
| Overall percentage (%) | 45% |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |
| Signature | Date    14/12/2016 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

   I.    the candidate's stated contribution to the publication is accurate (as detailed above);

   II.    permission is granted for the candidate in include the publication in the thesis; and

   III.    the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| Name of Co-Author | Jennifer L Marino |
|---|---|
| Contribution to the Paper | Consulted on the manuscript topic and structure and identification of co-authors. Contributed to the 'Limitations of existing literature' section of the manuscript. Contributed to critical review of contributions of co-authors. |
| Signature | Date    22/12/2016 |

| Signature | *Mare* (signature) | Date | 20/12/2016 |
|---|---|---|---|

| Name of Co-Author | Scott Davis | | |
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|---|---|---|---|
| Contribution to the Paper | Consulted on the manuscript topic and structure. Provided expert knowledge of perinatal epidemiology. Contributed to the 'Clinical Guidance for Shift Work and Preconception and Antenatal Care' section of the manuscript. | | |
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|---|---|---|---|
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| Signature | | Date | 21/12/2016 |

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| Signature | | Date | 14/12/2016 |

Please cut and paste additional co-author panels here as required.

## 2.4 Published manuscript

**Fixed or rotating night shift work undertaken by women: implications for fertility and miscarriage**

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**Abstract**

This review summarizes the evidence concerning effects of night shift work on women's reproductive health, specifically difficulty in conceiving and miscarriage. We distinguish between fixed night shift and rotating night shift since the population subgroups exposed, the social and biological mechanisms, and the magnitude of effects are likely to differ; of note, women working fixed night shift are known to have high tolerance for this schedule. We identified two relevant systematic reviews with meta-analyses and five additional studies. Night shift work may give rise to menstrual cycle disturbances, but effect sizes are imprecise. Endometriosis may be elevated in night shift workers, but evidence is only preliminary. Adequate data are lacking to assess associations between night shift work and infertility or time to pregnancy. The weight of evidence begins to point to working at night, whether in fixed or rotating shifts, as a risk factor for miscarriage. There are many methodological problems with this literature, with substantial variation in the definitions of night shift and schedule types making comparisons between studies difficult and pooling across studies questionable. Nevertheless, there appears to be grounds for caution and counselling where women have concerns about night shift work and their reproductive health.

**Keywords:** shift work, night shift work, fertility, pregnancy, miscarriage.

**Introduction**

Night shift work is relatively common among female workers. The industries in which women tend to be involved in night shift work are leisure and hospitality, health care, and transport and communication. As Western populations age, female participation in night shift work will grow with demand for services in health and aged care. Nursing, in particular, continues to have high rates of female employment, including many reproductive-aged women.[1]

As early as the 1970s, night shift work was suspected of affecting female reproductive health.[2] Difficulty in conceiving and miscarriage were of concern, and have continued to receive attention.

The definition of 'difficulty in conceiving' entails a judgement about how long it should normally take to achieve pregnancy. Conventionally, clinical infertility has been defined as the inability to become pregnant after 12 months of unprotected sex.[3] However, recent guidelines in the UK have signalled a change, with the recommendation that couples with unexplained infertility attempt natural conception for two years before being offered *in vitro* fertilization[4]; this reflects evidence that half of couples infertile at one year will conceive naturally if they continue trying for a further year.[5]

Difficulty maintaining pregnancy in the early stages is associated with a history of infertility but also occurs in other women.[6] For clinical purposes, miscarriage has been defined as delivery of an embryo or fetus prior to 20 weeks' gestation. After 20 weeks, fetal viability (with advanced life support) increases progressively, and between 25 and 28 weeks, most fetuses are considered to be viable, if very fragile. For some epidemiologic purposes, deliveries up to 28 weeks may be considered miscarriage (rather than very preterm delivery).

A number of mechanisms are hypothesized for potential associations between night shift work and reproductive health, described in more detail presently. Briefly, metabolic and hormonal disturbances could be induced by exposure to light at night, affecting the menstrual cycle (thereby fertility) or adaptation to

pregnancy. Metabolism could also be altered by insufficient or poor quality sleep following night shift work. Night shift work may predispose women to conditions associated with infertility and miscarriage, such as endometriosis.[7,8] Working at night may affect relationships and sexual behaviours, or health related behaviours that affect fertility, such as smoking and physical activity.

The aim of this review is to provide an overview of the evidence concerning the effects of work at night on women's reproductive health, specifically difficulties in conceiving and miscarriage. The focus is on hours in the paid workforce, but we acknowledge the large amount of unpaid labour and care work that many women undertake throughout the day.

**Defining and quantifying shift work**

Night shift workers may work the same shift all of the time (permanent or fixed) or rotate among different shift types (rotating shift). Rotating shifts typically contain a rest period between two blocks of working hours, or are characterized by working irregular 'as needed' shifts. Rotating shifts vary in the speed of rotation and the number and position of rest days within the shift work cycle.[9]

The definition of night shift (as distinct from evening or 'swing' shift) varies across countries, in terms of both the timing and duration of the work. Shift work has been defined as any work schedule outside of conventional daytime hours (9 am to 5 pm). In the US, night shift usually refers to work conducted between the hours of 9 pm and 8 am.[10] In Europe there is more variation, with some countries defining the night shift as a shift beginning 8–10 pm and finishing 5–7 am, and others a start time of 11 pm or midnight. Most European definitions specify at least three hours of duty.[9]

Comparisons of night shift work across countries are limited by differences in definitions and reporting. In the US, 16% of women report work that that includes evening, night or rotating shifts.[10] In the European Working Conditions Survey 2010, 17% of women reported undertaking shift work and 14% reported working at least one night shift per month.[11] In the US, the prevalence of night shift work

varies with age, with 20% of women aged 25 years reporting night shift work, compared to 11% of those aged 39 years; the lifetime prevalence of ever working evening or night shift is high, estimated to be 70%.[12]

It is important to appreciate that the ability to tolerate working at night varies between individuals. Tolerance, the ability to adapt to long-term shift work without adverse physiological consequences (usually characterized as sleep problems, fatigue, and digestive difficulties), has been related to gender, age, and circadian preference (chronotype, or morningness/eveningness).[13] It may also be influenced by situational and psychological variables. Working time control, giving employees a degree of autonomy over scheduling their shifts and off time, may increase shift work tolerance.[13-15] Those who work fixed night shift for extended periods are likely to have chosen this schedule and are a selected population with high tolerance of any acute social and metabolic consequences.[13,14] Those who work rotating shifts with nights may be more of a mixed group, including those with high tolerance and those lacking alternatives.

The above variability in definitions and the matter of tolerance portend problems in making comparisons between studies or interpreting pooled results.[16] Fixed night shift and rotating shift work that involves working at night are likely to have the greatest impact on circadian rhythms and reproductive biology. Thus we focus on these shift types in this review, considered separately wherever possible.

**Mechanisms through which night shift work may affect fertility and pregnancy**

Night shift work has been hypothesized to affect fertility and miscarriage by several mechanisms. Not all act directly on circadian or reproductive biology.

Indirect effects may operate through intimate relationships. For example, shift workers may have poorer relationships and increased family conflict compared with other workers,[17] although this is not inevitable.[18] Shift work could affect the timing of sexual intercourse with respect to ovulation, a crucial factor in the

probability of conception, as well as affecting intercourse frequency.[19] There is little literature about the social effects of shift work on time to conception.

Interruptions to daily routines can make it difficult to maintain healthy eating and exercise behaviours, which have salutary effects on fertility, partly through reduced obesity.[20] Many studies have reported altered eating habits in shift workers, including increased consumption of high energy snacks and other nutritionally poor foods.[21,22] In parallel, difficulties participating in structured sport and leisure activities combined with feelings of fatigue may alter activity patterns and energy expenditure. For example, Loprinzi et al.[23] observed reductions in moderate-to-vigorous physical activity of 59% among those working evening shift and 70% among those on night shift.

In the UK, smoking has been reported by 23% of women undertaking shift work, compared to 15% of other workers.[24] Though many women start smoking before entering the workforce, shift work has been linked to taking up smoking.[25] Smoking is associated with increased time to conception and early pregnancy loss.[26,27]

For many night shift workers, disruption of sleep patterns means that it is difficult to make up sleep and, when achieved, sleep may be of poor quality, lacking in slow-wave or non-rapid-eye-movement ('deep') sleep.[28] Insufficient or poor quality sleep has been shown to affect metabolism, including insulin resistance and glucose tolerance,[28] with acute and potentially longer-term implications for health.[29] Of relevance here, glucose homeostasis appears to be important for conception and establishment of pregnancy, [30] with an intricate balance of insulin and glucose needed for the embryo to survive and develop normally. Pre-implantation, low levels of glucose are essential for the embryo to be viable,[31] with either glucose excess or absence resulting in failure to progress.[32,33] Embryos collected from the oviducts or uteri of hyperglycaemic (diabetic) mice, then transferred to euglycaemic recipients, have an increased incidence of retarded fetal growth and fetal abnormalities,[34] which could translate to later miscarriage in humans. Consistent with these findings, women with type 1 or type 2 diabetes, or

'pre-diabetes', have elevated adverse pregnancy outcomes, including miscarriage and congenital abnormalities.[35,36]

Beyond insufficient or poor quality sleep, shift work disrupts the timing of many biological rhythms through being awake and/or exposed to light at night.[37-39] In turn, this creates discord within the individual's circadian timing system. Physiological systems are maintained in synchrony with the prevailing solar day via retinal light perception and transfer of the information to the suprachiasmatic nucleus (SCN) in the hypothalamus. Cells of the SCN maintain an endogenous 24 hour (circadian) rhythmicity via a cellular molecular feedback loop involving clock gene transcription factors. The SCN then informs peripheral organs (liver, muscle, pancreas, etc.) of the time of day through neural and hormonal pathways (e.g., melatonin and corticoids).[40,41] Peripheral organs including the ovary and uterus also maintain their own endogenous rhythmicity.[42] The integration of central and peripheral rhythmicity therefore allows diverse physiological functions to occur at specific and appropriate times of day or month, including ovulation and menstruation.[43,44]

The prenatal environment is inherently circadian. The developing fetus is exposed to fluctuating levels of temperature, substrates and hormones that oscillate over the 24 hour day, driven largely by the maternal system, through her endogenous behaviour and endocrine rhythms. The fetus gradually develops its own circadian system over gestation, with rhythms of heart rate, respiratory movements and hormone secretion readily detectable.[45-47] Animal studies have shown that disrupting maternal rhythmicity can have long term consequences for metabolic profiles of offspring.[48,49]

**Overview of epidemiological literature**

Two recently published systematic reviews including meta-analyses of available data have considered the effects of shift work on female fertility and pregnancy loss (Table 1).[50,51] Three studies published since are also summarized here.[52-54] No systematic reviews address endometriosis, so findings of the two primary pieces of research are presented.[55,56]

**Table 1: Summary of studies included in this review.**

| First author | Year of publication | Article type | Sample/studies | Exposure | Outcomes |
|---|---|---|---|---|---|
| Marino et al.[55] | 2008 | Primary research | Case-control study 235 cases 545 controls | Any evening shifts, any night shifts, percentage of time working evening or night shift, duration of shift work (years), sleep patterns | Laparoscopically-confirmed endometriosis |
| Schernhammer et al.[56] | 2011 | Primary research | Nurses' Health Study II cohort 89,400 women | Rotating night shift work (at least three nights per month), duration of rotating shift work (1–4 years, ≥5 years), sleep duration | Laparoscopically-confirmed endometriosis |
| Bonde et al.[50] | 2013 | Systematic review and meta-analysis | 5 studies 7 studies 52,032 women | Fixed night shift Rotating shifts including nights | Miscarriage (pregnancy loss before 28 weeks' gestation) |
| Feodor Nilsson et al.[52] | 2014 | Primary research | Registry-based cohort study 88,373 pregnancies | Fixed evening, fixed night, rotating shifts without nights, rotating shifts with nights, not working outside the home | Miscarriage (pregnancy loss before 22 weeks' of gestation) |
| Stocker et al.[51] | 2014 | Systematic review and meta-analysis | 15 studies 123,403 women | Shift work (any shift work outside the hours of 8:00 am to 6:00 pm), fixed night shift | Menstrual disruption (cycles <25 or >31 days) |

| | | | | | Infertility (time to pregnancy >12 months) |
| | | | | | Miscarriage (pregnancy loss before 25 weeks') |
| Gaskins et al.[53] | 2015 | Primary research | Subset of the Nurses' Health Study 3 cohort  1,739 women | Fixed evening shift, fixed night shift, rotating shifts with nights, rotating shifts without nights, frequency of night shift per week, duration of specific shift schedules (years) | Time to pregnancy (months) |
| Lawson et al.[54] | 2015 | Primary research | Nurses' Health Study 3 cohort  6,309 women | Fixed evening shift, fixed night shift, rotating shifts with nights, rotating shifts without nights, frequency of night shift per month, duration of night and rotating shift work (years) | Menstrual disruption including irregular cycles, short (<21 days) and long (≥40 days) cycles |

Bonde et al.[50] reviewed the outcome of miscarriage (pregnancy loss before 28 weeks of gestation) with a meta-analysis of data from seven studies. Rotating shift work (seven studies) and fixed night shift (five studies) were each compared to daytime work.

Stocker et al.[51] reviewed the outcomes of menstrual disturbance (short or long cycle), infertility (time to pregnancy), and miscarriage (pregnancy loss before 25 weeks of gestation) with meta-analysis of available data. Shift work was defined as work occurring outside the hours of 8 am and 6 pm and included fixed, rotating or mixed shift types. Reference groups were women working in the daytime or women who did not work outside the home. Separate subgroup analyses were presented for fixed night shift work, only, defined as a 10 to 12 hour shift beginning between 8 pm and 10 pm.

### *Menstrual disturbances*

Menstrual cycle length has been a focus of studies of shift work and reproductive health as it is considered a marker for subfertility.[57] In the systematic review of Stocker et al.,[51] pooled adjusted results indicated that women who undertook shift work of any type were 15% more likely to report altered menstrual cycle length (< 25 days or > 31 days). When analysis was limited to the two studies that considered fixed night shift work, the effect was magnified but no longer statistically significant (OR = 1.72, 95% CI 0.33–8.9). There was considerable heterogeneity between studies.

Since that review, menstrual disturbance has been investigated in a study of over 6,000 nurses and nursing students participating in the Nurses' Health Study 3.[54] Menstrual cycle characteristics of nurses working specific types of shift (evening only, night only, rotating shifts with nights, and rotating shifts without nights) were compared with those of (fixed) day time workers. After controlling for potential confounders (including some that arguably may be mediators, namely, smoking, body mass index, and physical activity) women working nights only or rotating shifts with nights had elevated occurrence of irregular cycles (OR = 1.32,

95% CI 1.15–1.51 and OR = 1.27, 95% CI 1.1–1.47, respectively). The number of night shifts performed per month was also associated with irregularity. There was some indication that rotating shifts with nights, but not other schedules, was associated with short (OR = 1.75, 95% CI 0.98–3.12) and long (OR = 1.28, 95% CI 1.03–1.61) cycle length. Neither evening shift nor rotating shift work without nights were associated with irregularity or cycle length. This study therefore supports the contention that rotating shifts with night work are, in practice, the most detrimental to fertility.

Overall, the evidence suggests that night shift work may give rise to menstrual cycle disturbances. Heterogeneity across studies means the magnitude of effects are imprecise. The types of schedule as well as the length of time for which a woman has undertaken night shift work may be relevant.

*Endometriosis*

Endometriosis is a female reproductive disorder associated with infertility. Its pathogenesis is thought to involve a combination of hormonal, immunological and inflammatory factors.[56,58] The overlap of these factors with the pathophysiological conditions produced by disruption of circadian rhythms has prompted research investigating a possible link between shift work and endometriosis.

In a case-control study, Marino et al.[55] investigated the association between shift work and laparoscopically-confirmed endometriosis and whether this association was modified by polymorphism in the human CLOCK (hCLOCK) gene (rs1801260). Any night shift work was associated with increased risk of endometriosis (OR = 1.48, 95% CI 0.96–2.29) and this was more pronounced for women who worked night shift more than 50% of the time in their job (OR = 1.98, 95% CI 1.01–3.85). This study also found a trend towards increasing risk of endometriosis with increasing duration of shift work (in years), although results were not statistically significant. hCLOCK gene polymorphism was not associated with endometriosis and did not affect the relationship between shift work and endometriosis, but other candidate polymorphisms remain plausible.

In a prospective study using data from the Nurses' Health Study II, Schernhammer et al.[56] found higher rates of self-reported laparoscopically-confirmed endometriosis among women who worked rotating night shift work (at least three nights per month) than among day workers, but only among those who had done shift work for at least five years and concurrently reported infertility (time to pregnancy > 12 months) (OR = 1.71, 95% CI 1.18–2.49). This effect was more pronounced among women with longer duration (five or more years) of rotating night shift work. (Data for fixed night shift work was not considered in this publication.)

Both studies also considered the role of sleep and sleep disturbances in the association between shift work and endometriosis. In the first, among women who worked more than 50% night shifts in their job, those who changed their sleeping time between shifts were more likely to report endometriosis, but the finding was not statistically significant, possibly reflecting the small number of women in this group.[55] The second found an association between sleep duration and endometriosis with women who slept on average for ≤ 5 hours or ≥ 9 hours having greater risk.[56]

Thus there is preliminary evidence suggesting that endometriosis may be elevated in women undertaking night shift work. Lack of uniformity in findings may mean some subgroups are more susceptible than others.

***Infertility or time to pregnancy***

In the review of Stocker et al.,[51] meta-analysis of data from five cohorts indicated that shift workers of any type were more likely than comparators to report infertility (time to pregnancy of more than 12 months), although this association was attenuated and not significant when adjusted odds ratios were combined. Only one study had specific data for fixed night shift work, reporting higher occurrence of infertility among workers with this schedule compared to non-shift-workers (OR = 1.72 95% CI 1.15–2.56).[51]

Time to pregnancy (in months) was investigated in the Nurses' Health Study 3,[53] with data obtained from 1,739 nurses who were employed outside the home and attempting to become pregnant. No association was found between time to pregnancy and any shift work schedule (evening only, night only, rotating shifts with or without nights) when compared to fixed daytime work. A variety of potential confounders including age, body mass index and smoking were taken into account.

Adequate data are lacking to assess associations between night shift work and infertility or time to pregnancy. No conclusions can be drawn.

*Miscarriage*

Both the systematic review of Bonde et al.[50] and of Stocker et al.[51] considered shift work and pregnancy loss, although with slightly different definitions of the exposure and outcome. Of the 12 studies included in the former, six were also included in latter.

Bonde et al.[50] demonstrated elevated occurrence of miscarriage among women who worked fixed night shift compared to those working day shifts (OR = 1.51, 95% CI 1.27–1.78). Stocker et al.[51] demonstrated elevated occurrence of miscarriage among fixed night shift workers compared to those working conventional hours or not working (pooled adjusted OR = 1.41, 95% CI 1.22–1.63). Bonde et al. did not find an association between miscarriage and a rotating three-shift schedule (OR = 1.12, 95% CI 0.96–1.30). There was no difference in occurrence of miscarriage among all shift workers combined, compared to others, but since distinguishing between shift types is important, this is not necessarily reassuring.

Subsequently, a study of the Danish National Birth Cohort investigated women's work schedules and the risk of miscarriage (pregnancy loss before 22 weeks of gestation) in 88,373 pregnancies.[52] Work schedule was defined as fixed daytime,

fixed evening, fixed night, rotating shifts without nights, rotating shifts with nights, and not working outside the home. Compared to daytime work, the risk of miscarriage was significantly higher among women who worked rotating shifts with nights (OR = 1.21, 95% CI 1.06–1.39). The risk for fixed night shift workers was elevated but not statistically significant (OR = 1.25, 95% CI 0.89–1.82), but this was a relatively small group. No other work schedules were associated with miscarriage. Results were adjusted for potential confounders including maternal age, parity, pre-pregnancy weight, exercise, smoking, alcohol and coffee consumption during pregnancy, and household occupational status.

Together, these epidemiological findings begin to point to working at night, whether in fixed or rotating shifts, as a risk factor for miscarriage, although it remains to be seen which is worse. Both systematic reviews found elevated risk associated with fixed night shift work, with an association of the same order of magnitude observed in the Danish study, albeit non-significant.[52] Neither systematic review implicated rotating shifts with nights, but results were pooled across studies with varying definitions of this schedule. The Danish study provides a basis for continued concern about rotating shifts with nights, warranting further research on a similar scale to assess consistency of the finding.

**Limitations of existing literature**

There are no established and recognised standard definitions of the exposure being studied. This makes comparisons between studies difficult, and pooling across studies questionable.
This point has been made strongly in relation to studies of night shift work and cancer.[16]

All observational studies of subfertility or adverse pregnancy outcomes are subject to certain systematic problems in measuring risk. Challenges of studying pregnancy include capturing intention to conceive and critical developmental windows for exposure effects. Chemical pregnancy and other very early pregnancy losses are often hidden events, and thus difficult to measure. In general

female populations unselected on reproductive intention, pregnancy loss is a relatively rare event, so studies must be large to capture sufficient events to detect differences. In addition, studies of shift work and female reproductive capacity are subject to three significant "worker effects" that may interfere with accurate investigation and interpretation.

The "healthy worker effect" is a selection bias in recruitment and retention arising because ill health reduces participation in the workforce.[59] Selective loss of those most affected results in underestimation of the association between exposures and outcomes. Of more relevance in the present context, the "infertile worker effect" arises because women raising families (i.e., in general, fertile women) spend less time in the workforce than their counterparts, so that employment may be inaccurately associated with reproductive disease.[60] Most importantly, the "healthy shift worker effect" refers to the tendency for those who are resilient to the negative effects of night shift work to remain in jobs with this schedule, thus artificially reducing any association between night shift work and adverse health outcomes; as mentioned, this is especially likely to be the case for fixed night shift, potentially masking important elevated risks.[61]

Although separate studies may compensate for these diverse sources of bias, they are likely to have a cumulative effect on attempts to synthesize the literature by meta-analysis or other methods. Thus it is likely to be difficult to obtain single interpretable risk estimates that are meaningful and useful to women, clinicians, employers and policymakers.

It is important to appreciate that there may be subpopulations of women for whom night shift work is particularly detrimental to reproductive health. We believe such subpopulations exist, given the suspected effects of psychosocial stress and sleeplessness on ovarian function,[62] and bearing in mind that not all women with poor tolerance to shift work can choose to avoid it. Women with other reproductive problems predisposing them to difficulties in conceiving and maintaining a pregnancy could also plausibly be at higher risk than other shift workers. For example, melatonin and cortisol disruptions could reasonably be

expected to exacerbate subfertility mediated by the hypothalamic-pituitary-ovarian-axis.

Another largely unmeasured influence on reproductive outcomes is access to prenatal care. Leaving aside economic issues, which vary greatly internationally and are currently in flux in the US, we do not know the effect of shift work rotas on scheduling and attending prenatal care clinics. Night and rotating shift workers might have greater free time during the (usually daytime) hours when clinics are available, but they might also attend at the cost of sleep, which can have deleterious effects on pregnancy.[63]

**Clinical guidance for shift work and preconception and antenatal care**

Laws in the UK and Europe require that employers put in place measures to reduce the risk of shift work to the health and safety of employees and offer alternative day employment or paid leave to workers with identified medical risks or at the employee's request.[64] Some countries specifically prohibit women from working night shift during pregnancy and the postpartum period.[9] The momentum for this appears to improvement in work-life balance and reconciliation between workers' professional and private lives, rather than the strength of the evidence linking night shift work with adverse reproductive outcomes.[65]

There are no evidence-based guidelines to assist women and their practitioners to translate the research on shift work and either fertility or miscarriage to application in clinical care. However, many reports suggest counselling or caution may be appropriate.

In relation to preconception care, an advisory report for the Dutch government (based on the two systematic reviews described above) concluded that there was no need at this stage to advise against shift work and night work prior to conception.[66]  In contrast, the UK National Institute of Clinical Excellence guideline for assessment and treatment of people with fertility problems identified shift work as an occupational hazard for hospital workers, associated with reduced

fecundability and prolonged time to pregnancy.[67] The guideline recommended that where individuals were concerned about their fertility, clinicians should enquire about occupation and offer 'appropriate advice'.

A UK guideline on the occupational aspects of pregnancy management by the Royal College of Physicians estimated that fixed night shift caused 6.1 (95% CI 3.2–9.4) extra miscarriages in every 100 pregnancies. The guideline recommended that pregnant women be informed that shift work may increase the risk of miscarriage slightly or not at all, and advice on changing work schedule should be tailored to each patient's tolerance and anxiety.[68] Narrative reviews and guidance documents by professional bodies such as the Royal College of Nursing,[64] the Health and Safety Authority in Ireland,[69] a UK trade union,[70] the International Labour Organisation Night Work Convention and Recommendation,[71] and the Australian Council of Trade Unions,[72] state that shift work, particularly night shift work, may be associated with menstrual cycle disruption or pregnancy complications including an increased risk of miscarriage.

**Conclusion**

Overall, the literature suggests that night shift work may give rise to menstrual cycle disturbances, but it is unclear whether effects are modest or substantial. Endometriosis may be induced or exacerbated, but evidence is only preliminary. No conclusions can be drawn from the limited data available regarding infertility. Evidence is accumulating that night shift work contributes to miscarriage but, again, the effect size is uncertain. Variation in key definitions as well as inability to separate vulnerable subgroups from other women may account for the blurriness of the evidence.

Further epidemiologic research is needed in which night shift work is characterised in detail to facilitate comparisons between studies and consolidation of findings from similar studies. Characteristics should include start and finish times of fixed or rotating shifts with nights, as well as how many nights are worked each month. Tolerance and the factors affecting it should be assessed.

Prospective studies are required to identify vulnerable subpopulations, ideally, following a cohort of young women before they commence night shift work through to family formation. Laboratory experiments to advance understanding of the molecular mechanisms underlying changes to circadian activity, in reproductive tissues, would be valuable.

Despite the limitations of current literature, we feel there are grounds for caution and counselling, especially where women are concerned that night shift work is affecting their reproductive health. Where women cannot request an alternative shift schedule, some practical steps may be possible. The research linking shift work with poor diet, reduced physical activity and smoking, reinforces the merit of counselling about health related behaviours. In addition, Smith and Eastman discuss in detail the following options to overcome circadian misalignment.[73] Exposure to bright light during night shift (especially blue light, if a light box is available) can enhance phase shift so that sleep the following day is of good quality. After night shift, sunlight should be avoided on the way home, through wearing dark sunglasses (especially those that block blue light), and the aim should be to go to bed as soon as possible in a dark room. Sunlight in the afternoon following sleep is also recommended. More broadly, to manage rotating shifts, tailored sleep plans can be made that, if strictly adhered to, can improve alignment of circadian rhythms.[73] These authors acknowledge, however, that it requires great co-operation from family and friends for some of these options to be viable.

We should also recognize that we have become diurnal animals over millions of years, so there are limits to what an individual woman can do to accommodate the physiological challenges presented by night shift work. Since night shift work is a 'probable carcinogen', with consistent evidence in relation to breast cancer,[9] there are already grounds for advocating that this schedule be used only when strictly necessary, with women given some control over scheduling wherever possible.

## Acknowledgements

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# 3 Project Two: Development of a job-exposure matrix (JEM) to assess shift work exposure among Australian women

This section outlines the background to the development of a job-exposure matrix (JEM) to assess shift work, with a focus on the mechanisms through which shift work may influence health. The key motivation for this project was the absence of an existing JEM that was appropriate for inferring exposure in epidemiological studies conducted in the Australian context.

Given that the data available in the subsequent studies contains only occupational title, a JEM provides an appropriate method for inferring shift work exposure. The JEM produced here was applied in further studies contained in this thesis to investigate the effects of night shift work and associated circadian disruption on fertility and fetal development.

This section begins with a review of occupational exposure assessment methodologies used in epidemiological studies. This is followed by a review of existing shift work JEMs and a discussion of the methodologies used in validating JEMs. Finally, the manuscript for the study, "Assessment of exposure to shift work mechanisms in the general population: the development of a new job-exposure matrix" is provided. This manuscript presents the results from a collaborative project to produce a shift work JEM for Australian women using data from a previous case-control study conducted in Western Australia.

## 3.1 Review of occupational exposure assessment methodologies

To investigate the relationship between occupation and a particular health or disease outcome (such as congenital anomalies in children) we must first consider the exposures that are potentially responsible for those particular outcomes. Once this has been clarified, it is necessary to establish which occupations are likely to involve these exposures. Occupational exposure can be assessed for epidemiological research in one or more of the following ways: using job title or code, a job-exposure matrix, self-assessed exposure and expert-assessed exposure. Biological testing and environmental monitoring provide an additional objective measure of exposure, however, their relative expense and resource intensity limits their use in epidemiological research, particularly when large sample sizes are required. Therefore, when assessing the merits of these methods for assigning occupational exposure it is important to also consider the time and resources required for each.[146]

**Job titles**

Job title information is often found in routine data collection registries, which provide a large sample for research at low cost. Job titles are usually given a numeric code corresponding to a systematic occupational coding scheme, such as the International Standard Classification of Occupations (ISCO). The use of registry data can overcome the problems of selection and recall bias, as it avoids participants self-selecting into a study based on their exposure or outcome experiences and exposure information is collected irrespective of outcome status. It also allows large scale analyses. Such analyses are frequently used in hypothesis generating studies, which aim to identify occupations at greater risk of mortality or morbidity.[147]

The use of job title allows comparison of health outcomes across occupational groups; however, job title alone does not give a complete indication of the exposures involved.[148] The major disadvantage is that there is no further

information regarding specific job tasks, dose and length of time exposed and this can lead to misclassification when assigning occupational exposure.[149]

**Job-exposure matrices (JEMs)**

Job titles may be used in combination with a JEM. Job-exposure matrices provide a cross-classification of job titles and occupational exposures and are often used in epidemiological research to retrospectively assign occupational exposures to study participants based on their job title.[149] Some JEMs include many occupations and exposures and are designed to investigate a wide variety of outcomes, for example the Finnish Job-Exposure Matrix (FINJEM) considers chemical, physical, microbiological, ergonomic and psychosocial agents,[150] whereas others are designed to investigate a specific exposure or category of exposure, such as occupational infections.[151]

Job-exposure matrices are usually composed of a job title axis and an exposure axis. The list of job titles to be included in the JEM may be coded according to a country-specific (e.g. Australian Standard Classification of Occupation (ASCO)) occupation coding system or an international system (e.g. ISCO). The exposure information may come from a variety of sources. Exposures may be assigned to job titles using information from published literature, from routine or special workplace monitoring, self-reporting by workers or using the knowledge and experience of experts in occupational hygiene.[149] In addition to indicating whether or not exposure is likely in a particular occupation, JEMs may also provide a semi-quantitative estimate of the level of exposure (such as high, moderate, low), the proportion of workers likely to be exposed in each occupation, industry information and the time period of exposure.[149, 152]

The advantages of JEMs include ease of use and cost effectiveness compared to other methods, such as expert assessment and in-depth interviews. JEMs can also overcome the problems of selection and recall bias, as participation does not rely on exposure or outcome status and assessment of exposure occurs independently of outcome status. The benefits of using JEMs must be weighed against the

disadvantages, which include subjectivity in exposure classification and an inability to consider differences in exposure levels within job categories. A JEM groups individuals with the same job title together despite potentially dissimilar working conditions. This misclassifies exposures non-differentially, with a tendency to bias risk estimates downwards towards the null (no effect) value. Further misclassification of exposure may also arise when translating occupational codes between countries.[153]

When using job titles or JEMs to classify exposure, occupations of interest must be chosen based on the strength of the association between occupational title and exposure. For some occupations there is a higher correlation between the job title, the tasks performed and likelihood of exposure to potential hazardous agents. For example, a welder, regardless of his/her specialisation is likely to be exposed to metal fumes. On the other hand, a laboratory assistant may or may not be exposed, depending on the particular chemical analyses they conduct as a part of their job.

**Expert assessment**

Expert assessment of occupational exposures usually involves a panel of occupational hygienists. These experts assign exposure probabilities and/or levels based on comprehensive information from job-history questionnaires or interviews with participants, or from expert knowledge gained through workplace inspection and monitoring.[154] Studies involving expert panels have been shown to have greater statistical power to detect associations between exposures and outcomes compared other methods, such as JEMs and self-reports.[155, 156] This reflects the greater accuracy in exposure classification that can be achieved with this method, and the associated reduction in misclassification.[148] However, the performance of expert panels relies on the quantity and quality of information that is available regarding job tasks and activities, the expertise of occupational hygienists concerning specific industries and occupations and the availability of up-to-date literature on the exposures of interest.[148, 154] Furthermore, expert panel

assessment is often expensive and time consuming because it requires experts to individually review each job.[146, 154]

**Self-assessment**

Self-assessed exposure can be collected by asking participants directly whether or not they are exposed to a list of target exposures in their job. This method can be implemented relatively quickly and cheaply, if participants are easily identifiable and contactable. The key limitation of self-reported exposure assessment is that cases and controls may differ in their efforts to recall past exposures and more generally, those who perceived their work to be more hazardous may overstate their exposure.[148] This can lead to reporting bias and therefore, misclassification bias.[148, 154] Difficulties may also arise when study participants are unaware of their workplace exposures, which is likely to be more relevant for chemical exposures where individuals may not know the specific type and concentrations involved. Further, self-reported exposure to physical hazards and shift work schedules, particularly night shift work, have been shown to be reasonably reliable.[157, 158]

Therefore, on balance, for large scale public health studies, JEMs represent a reasonable compromise between accuracy and resources. The key to their successful implementation being an adequate correspondence between the exposure of interest and occupational titles.

## 3.2  Review of shift work JEMs

A literature search identified five previously developed shift work JEMs. The JEMs included in this review are all general population JEMs, that is they have been developed to classify a variety of occupations across industries, not just occupations within a specific industry (industry-based JEMs). Two shift work JEMs were produce from expert assessment of self-reported job history information.[159, 160] A further two JEMs were produced using data from routine population surveys and data collections,[161, 162] and the final JEM combines data from multiple sources. [150] None of the JEMs were produced using Australian data.

Pronk et al.[159] developed a night shift work JEM using data from the population-based Shanghai Women's Health Survey. The JEM was created by an industrial hygienist using self-reported lifetime job histories obtained from the personal interviews conducted at baseline (1996–2000) and knowledge of local industrial conditions. Night shift work was defined as work beginning after 10pm at least three times in a month. The JEM classified jobs into four groups, no night shift, incidental night shift work, night shift that spanned only part of the night or on call work and work involving all-night shifts based on the likelihood of exposure to nigh shift work, never, low, median and high. The study did not consider rotating shifts.

The most notable hurdle to applying this JEM to Australian data is the differences in economic and industrial policies between China and Australia.[163] Occupations were also coded using a Chinese standard occupational and industry coding scheme, which may be difficult to translate into Australian occupational codes without local knowledge.

An American study of preterm birth, occupation and ethnicity also created a JEM using job information reported by participants in the University of California, Los Angeles Environment and Pregnancy Outcome Study.[160] Using this information, an industrial hygienist and a second reviewer, blind to participant status, classified exposure to shift work in each job, among other exposures, as either none, maybe or likely exposure.[160] The sample contained a high proportion of Hispanic women (over 50%). This limits the application of the JEM to Australia, as the Australian population is more ethnically diverse, and Hispanic would be a minority group. There are also likely to be socioeconomic differences between the two groups. The ethnicity and socioeconomic characteristics of the population in which a JEM is produced is important, as this may determine how accurately the JEM predicts the occurrence of shift work in professional jobs and jobs that require specialist qualifications, the prevalence of which may vary across population groups.

Three shift work JEMs have been produced in the European setting, two registry-based JEMs[161, 162] that look only at shift work, and FINJEM, [150] which has been produced by combining data from a number of sources and covers many different occupational exposures.

Hansen[161] produced a job-exposure matrix to assess female worker's exposure to night work and breast cancer risk in a Danish case-control study. Data from 2,603 women interviewed in the 1976 National Survey of Living and Working Conditions was used to identify trades in which at least 40% of female respondents worked predominantly night shifts. Shift work defined as a night time working schedule, although it is unclear how this was measured in the original survey. Night work was most common among unskilled workers and young women. Given that women who undergo fertility treatment in Australia tend to be older, of higher socioeconomic status and more highly educated,[7] this JEM may misclassify exposure if applied to Australian women.

The second European shift work JEM was produced in Sweden using data from population censuses and the annual Surveys of Living Conditions.[162] Shift work was defined as a rotating schedule with three or more possible shifts per day, or night work (any hours between 1:00 am and 4:00 am) at least one day during the week prior to the survey. The percentage of shift workers was calculated for each occupational category. In the subsequent study in which the JEM was applied, a 40% cut point was used to identify exposed occupations.[162] Like most JEMs, the Danish and Swedish JEMs have not been validated by comparing the JEM classifications to other exposure assessment measures (such as urinary melatonin) and neither appears to have been applied in other countries or settings.

The Finnish Job-Exposure Matrix or FINJEM is a database maintained by the Finnish Institute of Occupational Health (FIOH) which summarises information from other FIOH databases (such as the Register of Occupational Hygiene Measurements and the International Information System on Occupational Exposure to Carcinogens) and supplements them with information on the labour force and professional judgements.[161, 162] The FINJEM database contains

information on 311 occupations, 84 agents and eight periods of exposure (1945–2009).[150]

Estimates of night shift work in FINJEM were based on responses to the question on working time arrangements in the Quality of Life Work Survey 1990. Participants were asked to classify their work schedule as either 1) regular daytime work (6am–6pm), 2) two-shift work, regular evening or weekend work or other irregular hours that do not include night work, or 3) regular night work.[164] The FINJEM provides estimates of the proportion of workers exposed to night time work and the duration of exposure (years). This can be used to calculate a cumulative index of night time work exposure by multiplying the probability of exposure by the duration of exposure.[164]

Although FINJEM has been used in settings outside of Finland, reports of the applicability of FINJEM in other countries are mixed. The FINJEM has been modified for use in other countries, however these were all Nordic countries (Denmark, Iceland, Norway and Sweden) participating the in the Nordic Occupational Cancer Study (NOCCA).[165] This was feasible because the national occupational coding systems in these countries were either the same or similar, exposures were similar across countries, with few major differences and the economic structure of these countries is similar.[165]

The conversion of occupational codes is a major impediment to the application of FINJEM to Australian data. Previous studies that have applied the FINJEM outside of Finland have coded occupational titles using the international standard classification of occupation (ISCO) system. These codes were then matched to the Finnish occupation codes. Occupations in the current study are coded using the Australian Standard Classification of Occupation (ASCO). Translation of occupation from ASCO to ISCO to the Finnish codes adds uncertainty to the occupational classifications and increases the probability of misclassification bias.

When applying FINJEM (or in fact any of the JEMs reviewed here) to studies in other countries outside of this region (e.g. Australia), differences in work

practices, processes, technology and exposures between countries and over time should be taken into account, however this is difficult in practice.[154] It is important to note that, even after consideration of these economic and cultural factors and the validity of a JEM (discussed further in section 3.3), it is likely that a degree of uncertainty regarding the appropriateness of applying a JEM outside of its original context will remain. Thus, a more prudent approach may be to limit the application of a JEM to the purpose, time period and setting for which it has originally been developed.

## 3.3   Validation of JEMs

Validation of exposure assessment methods is an issue for occupational epidemiologists. As previously discussed, occupational exposure can be measured or assessed using a variety of methods, including self-reports, job specific questionnaires, expert assessment, job-exposure matrices (JEMs), quantitative measurement of biomarkers and environmental monitoring. While some of these methods are more precise than others, no one method can be considered a gold standard. The absence of a gold standard measure for many exposures means that conventional measures of validity such as specificity and sensitivity are of questionable utility, and that validity is often underestimated.[148]

Several studies have assessed the agreement between JEMs and other exposure assessment methods in an attempt to establish a sense of the JEM's validity. Bouyer & Hémon [166] suggest three main criteria to consider when studying the performance of JEMs. These are 1) Does the JEM accurately evaluate the exposure of interest? 2) How does the JEM perform statistically in terms of bias and power? 3) Can the JEM predict known associations between exposures and disease? These are outlined further, with examples, below.

**Does the JEM accurately evaluate the exposure of interest?**

Validation of JEMs centres on evaluating the performance of the JEM compared to some other method in terms of exposure classification.[167] In the absence of a

definitive gold standard for occupational exposure assessment, JEMs have been compared to quantitative data obtained from biological sampling or environmental monitoring, expert assessments of exposure, other JEMs and self-reported exposure. Despite the caveats mentioned above, correspondence between the JEM and other methods is often assessed using statistical measures of agreement such as sensitivity and specificity and the Kappa statistic.[166]

The previously described study by Ji et al.[163] measured the agreement between a JEM and urine sampling, providing an example of the use of biological sampling as a gold standard measure. As outlined above, the JEM was created by occupational hygienists using lifetime occupational history information to estimate the probability of working night shift work. Early morning spot urine samples from study participants were tested for concentration of 6-sulfatoxymelatonin (the primary metabolite of melatonin). The concentration of 6-sulfatoxymelatonin is predicted to be lower among night shift workers, whose exposure to light at night has resulted in decreased melatonin secretion. After adjusting for a number of lifestyle and reproductive factors, a significant inverse association was found between early morning urinary concentrations of aMT6s the likelihood of night shift work as classified by the JEM.[163] This indicates that participants who were classified as night workers by the JEM had lower levels of 6-sulfatoxymelatonin, giving some positive indications about the validity of the JEM.

The choice of urine sample testing as a gold standard involves certain caveats. Measurement of the concentration of solvent metabolites is prone to error, due to factors relating to the timing of sampling and metabolite half-lives, as well as inter-individual differences in metabolism. Although quantitative data potentially provides an objective measure of exposure, it is often not feasible in the context of a population based study.[168] Thus, researchers have looked to other reference measures when assessing whether a JEM accurately captures the exposure of interest.

Although not a study of shift work exposure, Solovieva et al. provide an example of an approach to the validation of a JEM using job-specific questionnaires as a reference method of exposure assessment.[169] This study compared a gender-specific JEM with job specific questionnaire-guided interviews in a Finnish study of occupationally-related lower back pain. Exposure prevalence thresholds of 50% and 40% where applied in the JEM for a set of six dichotomous exposure variables relating to physical work, and exposure estimates were only calculated for jobs with at least 20 individuals. Specificity values were reasonably good for both men and women, with values ranging from 0.84–0.92 and 0.91–0.98 respectively. However, sensitivity values were much lower, 0.18–0.55 for men and 0.13–0.0.42 for women.[169] Varying the exposure threshold from 50% to 40% was beneficial in terms of improved sensitivity without compromising specificity for the rarer exposures. However, for more common exposures gains in sensitivity were outweighed by a substantial decline in specificity.[169]

A similar approach was applied to assess the validity of the shift work JEM developed as part of this thesis. As there is no easily accessible gold standard for shift work, we compared the exposure classifications of the JEM with that of individual-level exposure determined via job-specific questionnaires completed by female participants in the Australian Work Exposure Study (AWES).[170] Sensitivity and specificity were calculated to evaluate the validity of the JEM. Further details of this analysis are provided in the published manuscript that follows this section.

**How does the JEM perform statistically, in terms of bias and power?**

By definition, a JEM involves the grouping of individuals by their occupation. The major consequences of this loss of information may be bias in the estimate of the odds ratio and/or a loss of statistical power to detect significant differences between exposure groups.[166] The magnitude of bias in the estimate can be quantified and corrected in the analyses. However, this requires that the sensitivity and specificity of the JEM are known, which as described above, is contingent on identifying an appropriate reference measure.

Assessing the effect of the JEM on statistical power is also complicated. The calculation of statistical power is relevant when the estimate produced using the JEM exposure classifications is unbiased or bias towards the null value. Bouyer and Hémon suggest the calculation of relative efficiency, to compare the sample size required (compared to the reference method) to achieve the same level of power. This requires knowledge of the population prevalence of exposure and may be difficult to interpret if both methods under consideration are considered biased, or no clear reference measure exists, which could be argued in most cases.[166]

In light of the difficulties in assessing this criteria, it was not considered when evaluating the validity of the shift work JEM developed for this thesis.

**Can the JEM predict known associations between exposures and disease?**

The final step discussed by Bouyer and Hémon in assessing the quality of a JEM involves testing its ability to reproduce known associations between risk factors and disease, either in terms of the existence or magnitude of the association.[166] It may be difficult to apply this approach in practice because the presence or absence of an association depends on more than exposure assessment. The sample size and sampling procedures, confounding and effect modification, as well as the distribution of exposure can also contribute to the likelihood of identifying an association.[166] Some of these caveats may be overcome by applying the JEM to the same study population from which the original association was obtained, which is the approach taken in the example below.

In their study of lower back pain (described above), Solovieva et al.[169] tested the ability of the group-based JEM to predict known associations between lower back pain and heavy physical work, heavy lifting, awkward trunk posture, arm elevation and kneeling/squatting, as determined by analysis of the individual-based data. For men, there was attenuation of the size of all five odds ratios, although the values remained statistically significant. For women, odds ratios

were attenuated for four out of five exposures (the odds ratio was increased for heavy lifting) and odds ratios for awkward trunk positions and arm elevation were no longer statically significant.[169] Attenuation of effect estimates when using a JEM is not unexpected. Use of a JEM tends to bias results towards the null, leading to an underestimation of effect size. This is because the misclassification of exposure produced when applying a JEM is non-differential, that is, it is independent of outcome status.[167]

This approach is also applied to assess validity of the shift work JEM developed for this thesis. The JEM was applied to the occupational data from which it was developed, the Breast Cancer Employment and Environment study.[171] Regression analyses were then conducted to determine the effect of group-based (JEM) exposure classification compared to the original individual-level exposure assignments on previously published risk estimates.[171]

## 3.4 Statement of authorship

# Statement of Authorship

| Title of Paper | Assessment of exposure to shiftwork mechanisms in the general population: the development of a new job-exposure matrix. |
|---|---|
| Publication Status | ☑ Published      ☐ Accepted for Publication <br> ☐ Submitted for Publication      ☐ Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | Fernandez RC, Peters S, Carey RN, Davies MJ, Fritschi L. Assessment of exposure to shiftwork mechanisms in the general population: the development of a new job-exposure matrix. Occup Environ Med. 2014;71(10):723-9. |

## Principal Author

| Name of Principal Author (Candidate) | Renae Clare Fernandez |
|---|---|
| Contribution to the Paper | Contributed to conception and design of the study. Coded the occupational history data and conducted the statistical analysis. Drafted the manuscript and acted as corresponding author. |
| Overall percentage (%) | 70% |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |
| Signature | | Date | 12/12/2016 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

   i.    the candidate's stated contribution to the publication is accurate (as detailed above);

   ii.    permission is granted for the candidate in include the publication in the thesis; and

   iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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| Contribution to the Paper | Contributed to conception and design of the study. Assisted with coding the occupational history data and critically reviewed the manuscript. |
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| Signature | | Date | 14/12/16 |

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## 3.5   Published manuscript

**Assessment of exposure to shiftwork mechanisms in the general population: the development of a new job-exposure matrix**

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**THUMBNAIL SKETCH**

| **What this paper adds** |
| --- |
| <ul><li>Shiftwork has the potential to affect the health of workers via a number of biological mechanisms.</li><li>Previous job-exposure matrices (JEMs) for assessing exposure to shiftwork focus only on exposure to night work or rotating shifts.</li><li>This paper describes the development of a JEM for assessing exposure to several variables that reflect biologically plausible mechanisms for the effect of shiftwork on health.</li><li>This JEM may provide an alternative method of exposure assessment in the absence of detailed job history and exposure data in general population studies.</li></ul> |

**Abstract**

**Objective:** To develop a job-exposure matrix (JEM) that estimates exposure to eight variables representing different aspects of shiftwork among female workers.

**Methods:** Occupational history and shiftwork exposure data were obtained from a population-based breast cancer case-control study. Exposure to light at night, phase shift, sleep disturbances, poor diet, lack of physical activity, lack of vitamin D, graveyard and early morning shifts, was calculated by occupational code. Three threshold values based on the frequency of exposure were considered (10%, 30% and 50%) for use as cut-offs in determining exposure for each occupational code. JEM-based exposure classification was compared to that from the OccIDEAS application (job-specific questionnaires and assessment by rules) by assessing the effect on the odds ratio (OR) for phase shift and breast cancer. Using data from the Australian Workplace Exposure Study, the specificity and sensitivity of the threshold values were calculated for each exposure variable.

**Results:** 127 of 413 occupational codes involved exposure to one or more shiftwork variables. Occupations with the highest probability of exposure shiftwork included nurses and midwives. Using the 30% threshold, the OR for the association between phase shift exposure and breast cancer was decreased and no longer statistically significant (OR = 1.14, 95% CI 0.92–1.42). The 30% cut-off point demonstrated best specificity and sensitivity, although results varied between exposure variables.

**Conclusions:** This JEM provides a set of indicators reflecting biologically plausible mechanisms for the potential impact of shiftwork on health and may provide an alternative method of exposure assessment in the absence of detailed job history and exposure data.

## Introduction

Shiftwork generally refers to the organisation of working hours such that different individuals work in succession, allowing work to continue beyond the typical eight hour day, and up to 24 hours.[1] Shift types typically include morning, afternoon, evening, and night, and can be further defined according to the worker's schedule of shifts - either the same shift all the time (permanent) or rotating in a clockwise/anticlockwise fashion.[2] Shiftwork is relatively common in developed countries. For example, among female workers, the prevalence of shiftwork was 17.2% in the European Union in 2005, 12.4% in the USA in 2004,[3] and 14% in Australia in 2009.[2] Australian industries with the highest proportion of females engaged in shiftwork were *Health Care and Social Assistance* and *Accommodation and Food Services* (both 32%), followed by *Arts and recreation services (24%)*.[2]

There has been interest in the adverse health effects associated with shiftwork particularly, the impact of night and rotating shiftwork on circadian rhythm, which is the 24 hour biological cycle that regulates sleep and wakefulness in humans, in synchrony with environmental stimuli such as light/dark, activity, and food intake.[4] Disruption of circadian rhythms can result in phase shift, which occurs when peripheral biological activities, such as digestion, become unsynchronised with the central sleep/wake cycle. Phase shift also alters metabolic activity and hormone secretion, which may contribute to long-term impaired metabolic health.[5]

Shiftwork related light exposure at night may also alter the secretion of the hormone melatonin, which is predominantly secreted by the pineal gland and is involved in the regulation of several physiological processes.[6] Under normal sleep/wake conditions, melatonin secretion is highest at night time. When exposure to light at night occurs, for example during night shifts, melatonin secretion can be reduced or shifted in timing.[7] Melatonin receptors are found in parts of the central nervous system and in peripheral organ systems including the female reproductive system. Alterations in endogenous melatonin production and

receptor expression have been implicated in a number of diseases including certain cancers, coronary artery disease and Alzheimer's disease.[8] Of note, a meta-analysis of 10 studies found an increased risk of breast cancer among female shift workers, with a dose-response relationship with duration of shift work.[9] Elevated risk of ovarian cancer has also been found among women working rotating shifts.[10]

These shiftwork mechanisms have also been shown to affect aspects of female reproductive health. Altered menstrual cycle length and cycle irregularity have been reported among nurses who work rotating shifts,[11] and regular night shift work has been associated with increased risk of endometriosis.[12] Permant night shift work has been associated with an increased risk of spontaneous abortion among nurses and other occupations.[13, 14]

Other mechanisms that could contribute to adverse health effects among shift workers include disruptions to the quantity and quality of sleep, which have been associated with impaired immune function and metabolism and may lead to fatigue, with the potential for increased risk of workplace accidents and injury.[15, 16] There are also concerns that permanent night shift workers are at risk of vitamin D deficiency due to lower exposure to sunlight.[17] Shift workers are also reported to have relatively poor diets, be less physically active, have a higher body mass index, and be more likely to smoke and consume alcohol at harmful levels.[18]

However, it is important to consider the opportunity for self-selection for shiftwork amongst those with greater physiological tolerance, or amongst those with psychological states which are better suited the work pattern. Either may induce a form of selection bias, or 'reverse causation'.

Shiftwork exposure can be ascertained via observation or surveying of workers, or via expert assessments. However, these methods usually require direct access to workers and may not be feasible for very large samples; therefore a job-exposure matrix (JEM) may be useful to impute exposures. A JEM is a cross-classification

of occupational titles or codes and exposures,[19] often using data from exposure studies, expert assessments, biological measurements, or environmental monitoring. A JEM may be constructed for a specific industry or for use among the general population, and depending on its structure may provide estimates of the probability, frequency and/or intensity of exposure for each occupational title. JEMs are often applied because of their ease of use and cost effectiveness, particularly in population-based studies, where information on occupational history is generally less detailed or when the size of the study makes other methods of exposure assessment less feasible.[20] Use of a JEM also allows standardized exposure assessment and reduces reporting bias, which may occur when the quality of the self-reported job histories and exposure information varies among participants.[21]

Assessment of shiftwork exposure in epidemiological studies is complicated by differences in the definitions of shiftwork, night shifts and rotating shifts applied across countries, industries and companies.[22] This has led to a range of metrics being used to capture the prevalence, duration and frequency of shiftwork schedules. Furthermore, while circadian disruption has been identified as a key mechanism for the detrimental health effects of shiftwork, particularly in relation to cancer, a clear definition of circadian disruption is yet to be established.[23]

Several JEMs exist for classifying shiftwork exposure among women.[24-26] The majority of these JEMs are industry-specific and focus only on exposure to night shift (yes or no), rather than the factors that potentially cause health effects. A mechanistic approach to shiftwork exposures on a biological basis can help to overcome differences in the definition of shiftwork and individual variation in ability to cope with shiftwork.[22] In light of the challenges in assessing shiftwork exposure, this paper presents a step towards the creation of JEMs with improved validity for linking occupations with shiftwork exposure among the female population. Our paper describes the development of a JEM in the general female population for assessing exposure to several variables that reflect biologically plausible mechanisms for the health effects of shift work.

**Methods**

*Source of exposure data*

The exposure data used to construct the JEM was obtained from the Breast Cancer, Employment and Environment Study (BCEES).[22] This population-based case-control study recruited women aged 18–80 years. Cases were women who were first diagnosed with invasive breast cancer between May 2009 and January 2011, and were identified from the Western Australian (WA) Cancer Registry. Age-matched controls were randomly selected from the WA electoral roll. Data collection for BCEES involved a mailed questionnaire followed by a telephone interview to assess occupational exposures. The questionnaire collected information relating to demographics, reproductive history, and lifestyle factors, as well as details on all jobs held for at least six months over the woman's working life. Data from 1,785 controls were used to construct the JEM in this study.

*Assessment of shiftwork exposures with OccIDEAS*

Participants who reported in their questionnaire that they worked shifts or had any job that was likely to involve shiftwork went on to complete a structured telephone interview containing a job-specific module. Participant responses were recorded in OccIDEAS, an online application which manages the interview process and occupational exposure assessment.[27] The interview questions included the type of roster (regular, varied, on call), whether they worked between the hours of midnight and 5am (graveyard shift), and whether they worked a shift that started between 5am and 7am (early morning shift). For jobs that involved more than one consecutive graveyard shift, further questions were asked to assess shiftwork exposures based on an *a priori* framework that was established to enable the assessment of potential health effects of shiftwork using biologically plausible mechanisms.[28] These questions related to exposure to light at night, phase shift, sleep disturbance, poor diet, lack of physical activity, and lack of vitamin D. These six mechanistic variables, as well as graveyard and early

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morning shifts, formed the exposure variables for the JEM. The use of alcohol to help sleep was also assessed; however it was omitted from the JEM as only 0.2% of participants reported exposure.

Using an inbuilt set of exposure rules, OccIDEAS provided automatic assessments of the probability of exposure to light at night, sleep disruption, poor diet, lack of physical activity, and lack of vitamin D for each of the jobs reported by the women interviewed. Exposure to phase shift was determined by manual review of the descriptions of shift schedules. The expert reviewers involved in this process were blinded to case-control status. For each of these variables, only participants with probable exposure were considered exposed in this study. The criteria used to establish probable exposure to each of the six mechanistic variables is outlined below.

Exposure to light at night was assessed by asking about the brightness of the light in the participant's normal working area during night shifts. Probable exposure was assigned for women exposed to bright or medium light in working areas and/or light in their bedroom when trying to sleep.

The phase shift variable was designed to identify patterns of shift work that produced desyncronisation of central and peripheral biological rhythms.[29] It was assessed by determining how many consecutive night shifts were worked, and the direction of rotating shifts, that is, backwards (night-afternoon-morning) or forwards (night-morning –afternoon). Probable exposure was assigned to women who worked two or more nights of forward rotation or three or more nights of backward rotation consecutively. These definitions were based on evidence, albeit mainly from animal studies, which show that the central cycle starts to adjust after several days, with adjustment being quicker during forward rotation.[30, 31]

Sleep disturbances were assessed by asking about the amount of sleep (hours) obtained between consecutive night shifts, the quality of sleep (extremely well to extremely bad), difficulties in falling and/or staying asleep, the use of medication to help sleep and light and noise in the bedroom when sleeping. Women who

experienced decreases in both quantity and quality were classified as having probable exposure to sleep disturbances.

Participants assessed the quality of their diet while on night shifts using a four-point scale ranging from very healthy (lots of vegetables and wholegrain cereals, fruit and some protein), to very unhealthy (mostly fatty and sweet foods). Participants whose diet was rated anything other than very healthy were considered exposed.

Physical activity was assessed by asking how many times per week the participant engaged in at least 20 minutes of vigorous exercise and at least 20 minutes of moderate exercise when working night shifts. Participants who exercised vigorously less than three times per week, or moderately less than five times per week were considered exposed.

Finally, vitamin D was assessed by asking about the amount of time spent outdoors between two consecutive night shifts. Probable exposure to lack of vitamin D was assigned to those who spent less than one hour outside.

### Coding of occupational history data

Job title, main duties and industry were collected as part of each BCEES participant's occupational history. This information was used to classify each job according to the International Standard Classification of Occupation 1968 (ISCO-68).[32] The coder (RF) was blind to the respondent's shiftwork exposure and disease status. Where there were difficulties in allocating a code, discussions were held between the authors to reach an agreement.

### Statistical analyses

To create the JEM, the proportion of BCEES workers who were probably exposed to each of the shiftwork variables (according to OccIDEAS) was used to produce an estimate of the prevalence of exposure for each occupational code. Three

threshold values for exposure were considered: 10%, 30% and 50%. These values represent cut-offs for assigning exposure to a particular occupational code. For example, using the 30% cut point, a specific occupation would be classified as exposed to light at night if at least 30% of workers in that occupational code had been assigned exposure to light at night. The JEM was then reapplied to the BCEES occupational data to assess the effect on the risk estimate for phase shift when using the JEM for exposure classification compared to the original individual-level exposure assignments. This analysis was limited to the phase shift variable, as this was the only statistically significant result observed in the BCEES analysis of shiftwork exposures and breast cancer.[22] Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression models, adjusted for age group.

In the absence of a gold standard for occupational exposure to shiftwork, we compared the JEM with the exposures assigned by OccIDEAS to individuals from a separate data set, the Australian Work Exposure Study (AWES).[33] AWES was a nation-wide cross-sectional telephone survey investigating the prevalence of current occupational exposure to 38 carcinogens, including shiftwork variables. Data collection for this study was carried out in 2011–2012, on a random sample of the population, reflecting the approximate distribution of the Australian work force by state and territory. Data were collected from 5,023 males and females aged between 18 and 65 who were currently in paid employment. The OccIDEAS application was used for data collection and exposure assessment (including shiftwork factors) in this study.[33]

Assessments of shiftwork exposure for the female AWES participants were made by applying the JEM to the job titles (coded to ISCO68). These exposure estimates were compared with those produced at an individual level by OccIDEAS based on the job-specific modules completed during the AWES data collection. Exposure prevalence was compared for the eight shiftwork variables described above. The assessments of exposure to these shiftwork variables by the JEM were evaluated by calculating sensitivity and specificity, in comparison with the OccIDEAS assignment of each job. This was done for each of the three cut-off

points for the JEM. Sensitivity and specificity were calculated using the Stata user-written command 'diagt'.[34] Occupation codes that appeared in the AWES data but not BCEES were excluded from this analysis.

All data manipulation and statistical analysis was performed using Stata version 12 (StataCorp, College Station, TX, USA).

**Results**

Of the 413 occupational codes present in the BCEES population, 127 involved exposure to at least one of the eight shiftwork variables. The highest prevalence of shiftwork exposure was found among occupations in ISCO68 Major Group 0/1: Professional, technical and related workers. The five-digit occupational codes within this group with the highest prevalence of exposure included specialised nurses and professional and auxiliary nurses and midwives. Relatively high prevalence of exposure was reported among some occupations in Major Group 5: Service workers, including nursing aides, and also in the supplementary major group containing armed forces personnel (Table 1).

**Table 1:** Occupational codes with at least 10 workers in which the prevalence of exposure to one or more of the shiftwork variables was 20% or more in a female study population (N=1 785).

| ISCO-68 Occupation Code | Count | Probability of exposure | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Major Group 0/1 Professional, technical and related workers | (N) | LN | PS | SD | PD | PA | VD | GY | EM |
| 0-14.90 Other physical science technicians | 18 | 0.167 | 0.222 | 0.111 | 0.222 | 0.222 | 0.111 | 0.111 | 0.333 |
| 0-61.05 General physician | 15 | 0.067 | 0.067 | 0.067 | 0.000 | 0.000 | 0.000 | 0.467 | 0.000 |
| 0-71.10 Professional nurse (general) | 465 | 0.542 | 0.504 | 0.222 | 0.497 | 0.417 | 0.260 | 0.637 | 0.159 |
| 0-71.20 Specialised nurse | 16 | 0.750 | 0.625 | 0.188 | 0.750 | 0.563 | 0.375 | 0.813 | 0.313 |
| 0-72.10 Auxiliary nurse | 223 | 0.592 | 0.610 | 0.224 | 0.574 | 0.475 | 0.350 | 0.659 | 0.251 |
| 0-73.10 Professional midwife | 98 | 0.806 | 0.786 | 0.429 | 0.765 | 0.541 | 0.449 | 0.959 | 0.316 |
| 0-74.10 Auxiliary midwife | 19 | 0.789 | 0.789 | 0.263 | 0.789 | 0.526 | 0.263 | 0.842 | 0.158 |
| 0-76.20 Physiotherapist | 13 | 0.231 | 0.154 | 0.154 | 0.231 | 0.077 | 0.000 | 0.231 | 0.000 |
| 0-77.10 Medical x-ray technician | 19 | 0.316 | 0.105 | 0.053 | 0.211 | 0.211 | 0.211 | 0.579 | 0.158 |
| Major Group 3: Clerical and related workers | (N) | LN | PS | SD | PD | PA | VD | GY | EM |
| 3-80.20 Telephone switchboard operator | 67 | 0.015 | 0.015 | 0.030 | 0.000 | 0.015 | 0.000 | 0.015 | 0.224 |
| 3-80.90 Other telephone and telegraph operators | 13 | 0.231 | 0.154 | 0.231 | 0.231 | 0.154 | 0.077 | 0.231 | 0.077 |
| 3-94.90 Other receptionists and travel agency clerks | 10 | 0.100 | 0.100 | 0.000 | 0.100 | 0.000 | 0.100 | 0.200 | 0.200 |
| Major Group 5: Service workers | (N) | LN | PS | SD | PD | PA | VD | GY | EM |
| 5-10.20 Working proprietor (hotel and restaurant) | 12 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.250 |
| 5-10.50 Working proprietor (café, bar, and snack bar) | 29 | 0.103 | 0.103 | 0.034 | 0.103 | 0.034 | 0.034 | 0.172 | 0.310 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5-31.20 Head cook | 13 | 0.077 | 0.077 | 0.000 | 0.077 | 0.000 | 0.000 | 0.077 | 0.538 |
| 5-31.30 Cook, except private service | 49 | 0.020 | 0.020 | 0.000 | 0.020 | 0.020 | 0.020 | 0.082 | 0.286 |
| 5-99.40 Nursing aid | 310 | 0.290 | 0.274 | 0.103 | 0.235 | 0.235 | 0.097 | 0.303 | 0.161 |
| Major Group 7/8/9: Production and related workers, transport operators and labourers | (N) | LN | PS | SD | PD | PA | VD | GY | EM |
| 9-85.40 Motor bus driver | 10 | 0.100 | 0.100 | 0.000 | 0.100 | 0.100 | 0.100 | 0.100 | 0.200 |
| 9-85.50 Lorry and van driver (local transport) | 29 | 0.034 | 0.069 | 0.034 | 0.034 | 0.034 | 0.069 | 0.103 | 0.310 |
| Supplementary major groups | (N) | LN | PS | SD | PD | PA | VD | GY | EM |
| Armed forces | 17 | 0.412 | 0.235 | 0.176 | 0.294 | 0.235 | 0.176 | 0.529 | 0.176 |

LN: Light at night, PS: Phase shift, SD: Sleep disturbances, PD: Poor diet, PA: Lack of physical activity, VD: Lack of vitamin D, GY: Graveyard shift, EM: Early morning shift

In BCEES, the original OR for phase shift exposure and breast cancer using the OccIDEAS exposure classification was 1.21 (95% CI 1.01–1.47).[22] Based on the JEM (30% cut-off point), the OR was reduced in magnitude and no longer statistically significant (OR = 1.14, 95% CI 0.92–1.42). Very similar results were obtained when using the 10% cut-off point (OR = 1.13, 95% CI 0.96–1.32) and the 50% cut-off point (1.14, 95% CI 0.91–1.42).

The second study, AWES, provided occupational data on 750 females and included 215 different ISCO-68 codes. Fifty eight job codes in AWES (representing 82 women) could not be classified by the JEM as these occupations were not reported by BCEES participants. Of these additional occupations, six were classified by OccIDEAS as having exposure to early morning work only or graveyard shift only. A further seven were classified as having exposure to one or more of the mechanistic shiftwork variables. All of these occupations contained few individuals, and only one contained more than one exposed individual (Supplementary Table 1).

Sensitivity and specificity values were calculated for each of the cut–offs, 10%, 30% and 50% using the AWES data (Table 2). For most shiftwork variables, the 30% cut-off point performed best in terms of specificity and sensitivity. Sleep disturbances, lack of vitamin D, and early morning shift were difficult to estimate. For these variables, the specificity using the 30% cut-off was above 80%; however the corresponding sensitivity values were particularly poor, at 9.8%, 57.5% and 17.5% respectively.

**Table 2:** Sensitivity and specificity of the shiftwork JEM assessments applied to the Australian Workplace Exposure Study data, compared to the original assessments.

| Shiftwork exposure variables | N* | Cut point | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|
| Light at night | 76 | 10% | 85.5 (75.6-92.6) | 68.1 (64.2-71.8) |
| | | 30% | 69.7 (58.1-79.8) | 78.6 (75.0-81.8) |
| | | 50% | 68.4 (56.8-78.6) | 80.1 (76.6-83.2) |
| Phase shift | 64 | 10% | 81.0 (69.1-89.8) | 66.6 (62.7-70.4) |
| | | 30% | 69.8 (57.0-80.8) | 77.7 (74.1-80.9) |
| | | 50% | 68.3 (55.3-79.4) | 78.2 (74.7-81.4) |
| Sleep disturbances | 61 | 10% | 73.8 (61.0-84.2) | 71.0 (67.2-74.6) |
| | | 30% | 9.8 (3.7-20.2) | 98.0 (96.6-99.0) |
| | | 50% | 1.6 (0.0-8.8) | 99.2 (98.1-99.7)) |
| Poor diet | 68 | 10% | 85.3 (74.6-92.7) | 67.7 (63.8-71.4) |
| | | 30% | 73.5 (61.4-83.5) | 80.2 (76.8-83.3) |
| | | 50% | 52.9 (40.5-65.2) | 86.7 (83.7-89.3) |
| Lack of physical activity | 52 | 10% | 86.5 (74.2-94.4) | 70.1 (66.3-73.7) |
| | | 30% | 76.9 (63.2-87.5) | 78.9 (75.5-82.1) |
| | | 50% | 23.1 (12.5-36.8) | 93.7 (91.5-95.5) |
| Lack of vitamin D | 47 | 10% | 78.7 (64.3-89.3) | 75.7 (72.1-79.0) |
| | | 30% | 57.5 (42.2-71.7) | 85.8 (82.8-88.5) |
| | | 50% | 2.1 (0.1-11.3) | 98.6 (97.3-99.3) |

| | | | | |
|---|---|---|---|---|
| Graveyard shift | 109 | 10% | 83.5 (75.2-89.9) | 66.2 (62.1-70.1) |
| | | 30% | 78.0 (69.0-85.4) | 75.5 (71.7-79.0) |
| | | 50% | 66.1 (56.4-74.9) | 81.6 (78.1-84.7) |
| Early morning shift | 117 | 10% | 73.7 (64.6-81.5) | 59.0 (54.8-63.2) |
| | | 30% | 17.5 (11.1-25.8) | 90.4 (87.7-92.8) |
| | | 50% | 0.9 (0.0-4.8) | 97.5 (95.8-98.6) |

[*]Number of exposed workers in the Australian Workplace Exposure Study

**Discussion**

We described the development of a JEM for the assessment of shiftwork exposures among women from a population-based case-control study, which assessed variables representing different aspects of shiftwork. These variables included exposure to light at night, phase shift, sleep disturbance, poor diet, lack of physical activity, lack of vitamin D, graveyard shifts, and early morning shifts. Of the 413 job titles reported by BCEES controls, 31% were associated with a non-zero probability of exposure to at least one of the shiftwork variables.

One measure of JEM quality is its ability to detect known associations.[35] We compared the OR for phase shift and breast cancer obtained from analysis of the original BCEES data (where exposure to shiftwork variables was assessed using automated expert assessment based on detailed job history information) with the OR obtained when exposure was assessed by applying the shiftwork JEM to the same study sample. Application of the JEM produced diluted ORs that were no longer statistically significant and quantitatively very similar across the 10%, 30% and 50% cut-off points. This suggests that the JEM has introduced non-differential misclassification that has biased the association towards the null.

Differences in the specificity of exposure definition between the variables may be a source of non-differential misclassification. Exposure estimates for variables

that are more objectively defined, for example, graveyard shift refers specifically to work between midnight and 5am, can be viewed with greater confidence than those that are more subjective, or influenced by individual behaviour and preferences. It is also expected that the potential for misclassification of exposure is lower for these objectively defined variables, however, as phase shift was the only variable to show a significant association with breast cancer in BCEES, we were unable to test the effect of the JEM on other variables.

From the comparisons of the AWES assessments by the JEM with the original assignments using OccIDEAS it appeared that the 30% cut point was most appropriate to estimate exposure to the shiftwork variables. Specificity was considered a more important measure of the validity of exposure assessment than sensitivity because occupational exposures tend to be relatively rare in the general population.[21] For five of the eight shiftwork variables, the 30% cut point for exposure produced the most acceptable level of specificity ($> 75\%$) without markedly compromising sensitivity ($>= 70\%$). The exceptions were sleep disturbances, lack of vitamin D, and early morning shifts. At the 30% cut point, specificity for sleep disturbances was 98.0% and the sensitivity was just 9.8%. Chronotypes, or individual variations in sleep/wake times, vary with sex and age and may contribute to difficulties in estimating sleep disturbances among shift workers.[36] Indeed, chronotype has been shown to modulate the influence of certain shiftwork schedules on the experience of sleep disturbances among rotating shift workers.[37]

Individual behavioural preferences, for example leisure time spent outdoors, may also explain the poor results obtained for vitamin D. However, this argument would also hold for variables such as poor diet and lack of physical activity, which produced fair specificity and sensitivity at the 30% cut point. The final variable which was difficult to estimate was early morning shifts. The poor sensitivity of the JEM for this variable could be explained by differences in the time periods for which occupational information was collected between AWES (current job only) and BCEES (complete job history). Changes in working hours, organisation and conditions over time have possibly produced changes in the types and number of jobs that involve early morning work. There were 105 jobs

involving early morning work in BCEES and only 24 of these corresponded with the jobs in AWES that reported early morning work. Possible changes in working time arrangements are also relevant when considering why poor sensitivity was apparent for the early morning shifts but not graveyard shifts. It is possible that the latter, unlike the former have remained relatively stable over time. Again this is supported by the data, which showed that all of the 48 BCEES jobs reporting graveyard work match up with the jobs in AWES that reported graveyard shiftwork.

Shiftwork JEMs have been created from routine surveys,[24, 38, 39] or from expert assessments of job histories.[25, 40] For JEMs created from routine data, the definitions of shiftwork exposure varied from involvement in night time working schedules,[24, 38] to working a rotating schedule with three or more possible shifts per day, or having work hours during the night (any hours between 1am and 4am) at least one day during the week prior to the survey.[24, 39] When applying JEMs created from routine surveys, studies used relatively high cut points (over 40%) in an attempt to diminish misclassification of the non-exposed.

For two shiftwork JEMs that were created using expert assessment of job histories, the authors provided comparisons of the JEM classifications to other exposure assessment methods. Pronk *et al.*[40] compared the JEM classifications to self-reported exposures, finding a higher prevalence of night shift work using the JEM (44% ever exposed to nightshift work) compared to self-reports (26%). Ji *et al.*[25] compared a JEM assessment of night shift work to urinary concentrations of 6-sulfatoxymelatonin, the primary urinary metabolite of melatonin that is increased after a normal night of sleep. A significant inverse association was found between the nightshift JEM scores and urinary 6-sulfatoxymelatonin levels in early morning samples, providing some evidence to support the JEMs validity in this population.[25]

These existing shiftwork JEMs focussed on the assessment of the probability of exposure to night shift work, rather than the more specific characteristics of shiftwork which may be the causative factors for health effects, working either in isolation or in combination.[28] For example, night jobs may involve working

primarily in dark environments such as outdoor security work, dim environments such as hospital wards, or very bright environments such as airports or operating theatres. Hence, there may be substantial difference in variables such as the aggregate hours of exposure to bright light, and the number of bright light periods per 24 hours. Inconsistencies and broadness in the definition of shiftwork has been identified as a limitation of existing epidemiological literature, particularly in regard to studies of shiftwork and cancer.[3] In order to overcome complexities in uniformily defining and assessing shiftwork exposure, an approach that considers the biological mechanisms through which shiftwork effects health is warranted. As such, the development of this JEM, which considers several biologically plausible mechanisms will not only enhance understanding of the mechanism by which shiftwork produces ill health, but also provides a standard set of indicators which can be employed in future studies.

Despite the potential benefits of JEMs for population-based studies, the limitations of this approach are noteworthy. A JEM cannot account for variability of exposure within job codes. It is known that occupational exposures can vary between workers employed in the same job, even in the same location.[41] This suggests that individual behaviour is an important determinant of exposure and a determinant that is not adequately captured by JEMs. This may be particularly relevant to some of the variables in our study that are highly dependent on personal behaviours, such as poor diet, lack of physical activity, and lack of vitamin D and may contribute to the misclassification observed when applying the JEM to other data.

In addition, the shiftwork JEM presented here has been produced using data obtained from a study of Australian women. A number of ISCO-68 codes were not reported by participants in BCEES and therefore exposure information was missing. Many of these occupations tend to be male-dominated and it is likely that some would be very rare in general. Furthermore, many of these jobs would be unexposed to shiftwork, so their exclusion from the JEM is not of great concern. Regardless of these points, it may not be appropriate to apply the JEM in male populations and the frequency of shiftwork exposures in predominately male occupations may not be estimable.

It should also be noted that some occupational codes included in the JEM contain very small numbers and therefore the probability of shiftwork exposure for these codes should be viewed with caution (Table 1). We are more confident in the exposure estimates for occupations with greater n-values, compared to less common jobs. Lastly, it is possible that coding errors in assigning ISCO-68 codes to occupational data could contribute to misclassification of exposure.

These caveats will need to be taken into account when applying this JEM to other data in future studies and researchers are encouraged to carefully review the exposure assessment. This is particularly important when applying this JEM to study populations in other countries. Researchers are advised to manually check those jobs not captured by the JEM, with a clearly defined rule for assigning exposure such as using the hierarchical structure of ISCO. Researchers are also advised to double check the exposure classification for jobs that are common in their study population.

In a study investigating the applicability of a British JEM in a Finnish population, Kauppinen et al.[42] found that the British JEM performed satisfactorily for common exposures, that is, those with a prevalence of at least 10%. Rules for exposure assessment may also vary depending on differences in the industrial environment and processes between countries. The prevalence of exposure and the applicability of this JEM in other populations or countries are likely to be influenced by the economic structure, sex and age distribution of that population.[42] Researchers are also advised to consider the effect of changes in working conditions over time and the influence this may have on the applicability of some of the JEM variables (particularly the early morning shifts) to their study. This also extends to changes in the types of jobs that women are involved in and the expansion of female workers in to industries that were traditionally dominated by male workers.

For future JEMs of this kind, we recommend the development of more objective definitions of exposure for the shiftwork variables, to reduce the potential for misclassification due to individual preferences and interpretations of exposure.

Despite these caveats, our JEM is likely to provide an alternative means of assessing exposure to shiftwork related variables in the absence of detailed job histories and exposure data. The shiftwork JEM provides a useful tool for future studies as it provides a standard set of indicators that reflect biologically plausible mechanisms for the potential impact of shiftwork on health.

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## Competing interests

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**Supplementary Table 1:** Occupational codes involving shiftwork reported by female AWES participants that could not be classified using the JEM, because these jobs were not reported by BCEES participants.

| ISCO-68 Occupation Code | Total (N) | Number exposed to each shiftwork variable | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | LN | PS | SD | PD | PA | VD | GY | EM |
| 0-23.30 Power distribution and transmission engineer | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 0-26.30 Physical metallurgist | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 0-34.90 Other electrical and electronics engineering technicians | 3 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| 0-43.00 Ships engineer | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| 0-53.30 Horticulturist | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1-33.20 First level education teachers | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1-93.40 Social worker (delinquency) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1-93.90 Other social workers | 3 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3-00.10 Clerical supervisor (general) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 3-59.40 Road transport supervisor | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| 3-93.20 Correspondence clerk | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 7-11.05 Miner (general) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 9-69.50 Water treatment plant operator (water-works) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |

LN: Light at night, PS: Phase shift, SD: Sleep disturbances, PD: Poor diet, PA: Lack of physical activity, VD: Lack of vitamin D, GY: Graveyard shift, EM: Early morning shift.

# 4  Construction of the South Australian Birth Cohort

This section describes the construction and preparation of the dataset used for analyses in projects three and four.

**Data sources**

The South Australian (SA) Birth Cohort was produced by linking data from three sources: clinical data from the two ART clinics providing care in SA at the time; perinatal data and birth defects data from the SA Department of Health for the period 1986–2002 (Figure 3).

Data on treatment with ART was provided by the two clinics in South Australia (population 1.6 million) licensed at the time to provide fertility treatment involving embryo manipulation. The clinics (established by the University of Adelaide and Flinders University) provided data for all patient visits for fertility treatment for the period 1986–2002, creating a complete state-wide record of all clinical treatment involving ART.

When pregnancy occurs following fertility treatment in Australia, the outcome must be recorded according to a uniform protocol required by a national Reproductive Technology Accreditation Committee (RTAC). This enables potential linkage of patient data to the perinatal outcomes databases within a state jurisdiction.  To reduce the risk of ascertainment bias, patients with a residential address outside of SA were excluded in creation of the cohort (<0.5% of the sample).

The ART clinical data were linked to the state-wide perinatal collection, which requires by law the notification of all live births and stillbirths of at least 20 weeks gestation or 400 grams birthweight in SA by hospital and homebirth midwives using a standardised notification form (website www.health.sa.gov.au/pehs/pregnancyoutcome.htm). Notifications of all medical

terminations of pregnancy are also required by law and those that are induced at 20 weeks gestation or more are included in the perinatal data collection. These perinatal records contain information on maternal pre-existing conditions and medical conditions and complications during pregnancy. Other information also provided to the registry includes maternal demographic and lifestyle information such as age, postcode, smoking history (from 1998), and BMI (from 2003).

Birth defects are notifiable to the SA Birth Defects Register up to a child's fifth birthday. Structural, biochemical, chromosomal and other genetic defects are included and classified by registry staff according to the *British Paediatric Association Modification of the International Classification of Diseases 9th Revision* (ICD-9 BPA). Minor defects are excluded except where they are disfiguring or require treatment. A full list of the defects included and excluded can be found in Davies et al. (supplementary data).[7] Coding of birth defects occurs independently of birth defect notifications; however blinding of mode of conception among clinicians issuing the notifications was not possible. Assessment of this reporting method in a validation sub-study of birth defect ascertainment using a blinded clinical assessor did not indicate significant reporting bias.[172]

Data linkage was performed by the SA Department of Health. A unique number assigned to each delivery was used to link ART clinic and Birth Defects Register data to the state Perinatal Collection data. Linkage of ART data was conducted using probabilistic matching software (Automatch V4.3, MatchWare Technologies) and hand matching using patient identifiers and birth outcome data.

Extensive preparation of the dataset was then undertaken. This has included checking the veracity of computer-generated links between ART pregnancies and perinatal outcome data, by assessing agreement between key variables common to both datasets (e.g. parity, birth order) for each linked record; incorrect links were manually corrected. In addition some ART pregnancies were known to have resulted in a birth but were not automatically linked, so manual searches for candidate links were undertaken. Sequential births to individual mothers were compiled using a mixture of data management programming using the history of

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previous births within the health data, and manual searching for siblings where there were data errors (based on maternal parity and reported dates of previous births). This was necessary because in the perinatal dataset, the unit record and accession number relates to births, and so no unique identifier exists for linking sequential births to individual women. Links with Birth Defects Register data were also checked and corrected. Data linkage was completed by 2008.

The data linkage project described above was reviewed and approved by ethics committees at Flinders University, University of Adelaide and the SA Department of Health in 2005. Individual-level consent was not required.

The SA Birth Cohort, resulting from this data linkage process contains information on 319,038 naturally conceived births and 6,178 ART births. ART births by treatment modality are provided in Table 2. The linked dataset also contains information on possible confounders including: maternal age, parity, fetal sex, year of birth, maternal ethnicity, maternal country of birth, maternal conditions in pregnancy, maternal smoking in pregnancy, postal code indicators of socio-economic status and maternal and paternal occupation.

**Table 2:** Fertility treatment births by treatment modality.

| Treatment modality | Births (n) |
| --- | :---: |
| Minimal intervention (e.g. cycle tracking) | 725 |
| Ovulation induction | 427 |
| IUI | 734 |
| GIFT | 585 |
| IVF | 2,220 |
| ICSI | 1,399 |
| Donor oocyte | 88 |
| **TOTAL** | **6,178** |

**Infertility diagnosis and fertility treatment type data cleaning**

Where a fertility diagnosis was not explicitly given in the ART clinic data or where the treatment received did not match with the diagnosis provided, rules for assigning a diagnosis were established, drawing on a range of available

information, and implemented programmatically. The information available in the dataset to assist with this process included the results of diagnostic testing, such as blood tests, semen analysis, laparoscopy and ultrasound scans. Where a classification could not be derived programmatically, cases were examined individually. In some cases there were simple data entry errors and the missing information was located in other variable fields.  In more complex cases (e.g. use of donor oocytes in the absence of any diagnosed female infertility), other variables including medical conditions, medications and diagnostic test results, and the relevant published literature were examined to determine the appropriate fertility diagnosis.

In addition to binary infertility diagnosis variables, of which several could apply to any one couple, an augmented infertility diagnosis was created to summarise the main diagnosis for each couple. Each couple has one augmented infertility diagnosis, which could be either 'male infertility only', 'tubal infertility only', 'ovulatory infertility only', 'endometriosis only', 'other or mixed female infertility', 'combined male and female infertility', 'idiopathic' or 'missing'.

The infertility clinic data classified the treatment type into over 80 different categories. These categories were consolidated into 11 categories (nine listed in Table 2, plus spontaneous during/after treatment and infertile untreated) to simplify the use of this variable in analyses and reporting. Appendix 2 contains a list of the original and revised fertility treatment codes and a key to how to categories were consolidated.

**Figure 3:** Diagram summarising the data sources and data linkage process for construction of the South Australian Birth Cohort.

**ART Clinic Data**

- 6,178 ART births (4,107 primiparous)
- Clinical infertility investigation and diagnosis
- Reproductive and medical history

**State Perinatal Collection**

- 319, 038 live births and stillbirths >20 weeks gestation, medical terminations (127,700 primiparous)
- Maternal pre-existing conditions, conditions and complications in pregnancy and labour
- Age, ethnicity, postcode, title of usual occupation (ASCO coded), smoking (from 1998)

**SA Birth Defects Register**

- Detected at termination / birth or neonatal period
- Plus notifications from multiple sources up to five years of age
- Structural, biochemical, chromosomal, other genetic
- British Paediatric Association modification of ICD 9 codes

Data linkage via unique accession number for each birth

**South Australian Birth Cohort**

(1986 – 2002)

Data linkage via Automatch software and hand matching using patient identifiers and birth outcome data

# 5 Project Three: Night shift work, fertility treatment and infertility diagnoses

## 5.1 Introduction

Project three describes a series of analyses that aim to identify whether night shift workers are more likely to access fertility treatment to conceive their first pregnancy compared to their non-shift working counterparts and to determine whether this is related to differential infertility diagnoses. Following this introduction, the section includes a brief rationale for this investigation, and the manuscript presenting the study.

As described previously, night and rotating shift work are more likely to produce circadian disruption and phase shift.[136] Thus, the exposure of interest in projects three and four is exposure to light at night, for which exposure to shift work involving night and rotating shift work is a proxy, referred to as 'night shift work' from this point forward.

**Rationale**

Epidemiological studies have previously investigated the effects of night shift work on female fertility. These have focussed on menstrual irregularity and disturbances, time to pregnancy,[94] or endometriosis.[103, 105] However, no study has investigated the association between night shift work and the use of fertility treatment to conceive in the general population. Additionally, aside from menstrual irregularities and endometriosis, it is not known whether night shift workers are at greater risk of other forms of subfertility, including ovulatory dysfunction.

The specific research questions guiding this analysis are:

1. Are women employed in occupations with probable exposure to night shift work more likely than others to access a fertility clinic to conceive their first child?
2. Do those receiving clinic treatment have distinctive patterns of infertility diagnosis?

**Dataset for project three**

A woman-level dataset was created from the SA Birth Cohort for this project. Each woman was included once, representing her first pregnancy of >20 weeks gestation. It is beyond the scope of this project to consider women who sought fertility treatment to conceive but did not become pregnant or did not maintain a pregnancy for 20 weeks. Occupational information for these women was not collected consistently by ART clinics and was not coded using ASCO, so is not readily available for analysis.

Restricting analysis to first pregnancy or birth has a long history in research on fertility. This is appropriate when an exposure is potentially harmful to reproduction and past pregnancy affects the present likelihood of exposure.[173] In South Australia, many women having a second (or later) birth, with a usual occupation that features night shift work recorded in the perinatal registry, will actually be working part-time or not in the paid workforce, so they can care for their firstborn.[174]

Recently there has been discussion of parity-conditioning bias,[175] a form of selection bias that can arise when a cross-sectional study is based on a population with a wide age-range that is nulliparous at a specific point in time. This has the consequence that relatively infertile women predominate among the older women, especially when others of their generation typically had a first birth when aged in their early 20s. This bias is especially relevant in historical studies of time to pregnancy; ideally, these studies should consider reproductive success of entire birth cohorts from menarche to menopause.

The present study is not affected by parity-conditioning bias because it is not concerned with time trends in prevalence of a fertility characteristic. Instead it is concerned with an association between occupational exposure and mode of conception. Thus, while the oldest women included in the study (born in the 1940s and 50s) may be relatively infertile, they have not been selected in a way that would artificially create a connection between occupations involving night shift work and use of ART to conceive. In the youngest women, recourse to ART is uncommon, but again the focus is not on the extent of ART use, but rather associations with an aspect of occupation.

The majority of women classified as exposed to night shift work were employed as nurses (registered or enrolled). This is potentially a concern in research investigating the uptake of fertility treatment, as nurses may be differential users of health care services and may be more accepting of medical intervention for infertility compared to other women. It is not possible to gauge this with available data. However, nurses were a minority of women in paid employment who conceived a first birth with ART, comprising 5–16% in any given year (Figure 4).

**Figure 4:** Births conceived by nurses using fertility treatment as a percentage of all births from fertility treatment by year.
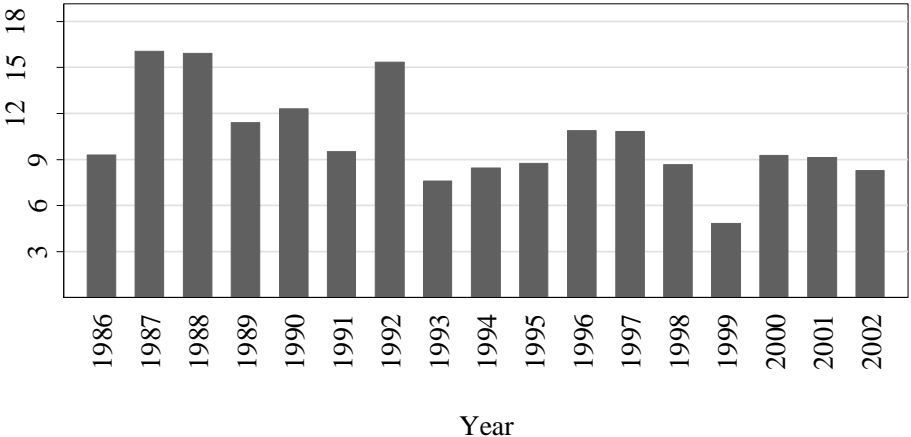
## 5.2 Statement of authorship

# Statement of Authorship

| Title of Paper | Night shift and women: is it associated with difficulty conceiving a first child? |
|---|---|
| Publication Status | ☐ Published      ☐ Accepted for Publication<br>☒ Submitted for Publication      ☐ Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | Fernandez RC, Moore VM, Marino JL & Davies MJ. Night shift and women: is it associated with difficulty conceiving a first child? Scandinavian Journal of Work, Environment and Health. Submitted 7 December 2016. |

## Principal Author

| Name of Principal Author (Candidate) | Renae Clare Fernandez | | |
|---|---|---|---|
| Contribution to the Paper | Assisted with cleaning of data for analysis. Contributed to conception and design of the study. Planned and conducted the statistical analysis. Contributed to interpretation of the research data. Drafted the manuscript and acted as corresponding author. | | |
| Overall percentage (%) | 80% | | |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. | | |
| Signature | | Date | 8/12/16 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

    i.    the candidate's stated contribution to the publication is accurate (as detailed above);

    ii.    permission is granted for the candidate in include the publication in the thesis; and

    iii.    the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| Name of Co-Author | Vivienne M. Moore | | |
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## 5.3 Manuscript

**Night shift among women: is it associated with fertility treatment to conceive a first birth?**

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**Abstract**

**Objectives:** The aims of this study were to investigate whether women who worked night shift disproportionately required fertility treatment to conceive a first birth and whether specific diagnoses were implicated.

**Methods:** In a retrospective cohort design, courses of fertility treatment resulting in a birth were linked to the state perinatal registry for South Australia for the years 1986-2002. Night shift work was used as a proxy for exposure to light at night and this was imputed from usual occupation by a job-exposure matrix. Using logistic regression the association between night shift work and use of fertility treatment was assessed among primiparous women in paid employment, then among all primiparous women. Next, among those who received fertility treatment, infertility diagnoses were compared according to night shift work status. Potential confounders were considered.

**Results:** Night shift working women (n=11,000) were more likely to conceive their first birth with fertility treatment compared to women in paid employment who did not work night shift (OR=1.25, 95% CI 1.09-1.43). Ethnicity and socioeconomic status did not affect this result. This result was attenuated when adjusted for age (OR = 1.10, 95% CI 0.95-1.26). Among primiparous women receiving fertility treatment, night shift workers were more likely than other women in paid employment to have menstrual irregularity (Adjusted OR=1.42, 95% CI 1.05-1.91) or endometriosis (Adjusted OR=1.34, 95% CI 1.00-1.80).

**Conclusion:** Night shift workers may have elevated need for fertility treatment as a consequence of an excess of menstrual irregularity and endometriosis. Older age at first birth may independently contribute to the need for fertility treatment but also accrues due to difficulty conceiving.

**Introduction**

The nature of paid work and the workforce in Western societies is changing, with manual labouring jobs declining and demand for workers in the service and care industries increasing. One implication of this is increased non-standard and flexible working time arrangements (1). Such changes in work arrangements disproportionately affect women, who predominate in the growth industries (2).

Night shift work, in particular, may disrupt the lives and health of women, with plausible effects on reproductive biology. Mechanisms include lifestyle, metabolic and immunological disturbances. Quantity and quality of sleep can be affected and the circadian rhythm, the 24-hour biological cycle that regulates sleep and wakefulness, can be perturbed (3). Circadian activity is co-ordinated by the suprachiasmatic nucleus in the hypothalamus which relays information from environmental stimuli to other parts of the brain and peripheral organs (4, 5). Asynchrony in circadian processes alters many physiological systems, including female reproduction (6, 7). Fixed night shift and rotating schedules including night shift are of most concern as they are thought to have the greatest impact (3).

Consequences of night shift work for female reproductive health, investigated in epidemiological studies, have included disturbed menstrual cycles (8), disorders such as endometriosis (9, 10), and long time to pregnancy (11). To our knowledge, no study has investigated the potential relationship between night shift work in the general population and need for fertility treatment to conceive. The aim of this study was to investigate whether primiparous women employed in occupations involving night shift work were more likely than women in occupations not involving night shift work to require fertility treatment and, if so, to characterise infertility diagnoses.

**Methods**

*Data sources and study population*

As described previously (12), the cohort for this study was assembled using data from the South Australian perinatal registry for the period January 1986 to December 2002. By law, all live births and stillbirths occurring after 20 weeks' gestation in South Australia must be reported to this registry.

Data relating to patients undergoing assessment and treatment for infertility were obtained from the two clinics that were licensed at the time to provide treatment involving manipulation of gametes or embryos. This also included data on patients who received less invasive treatment within the clinic setting, including treatment with ovulation induction drugs only. Fertility treatment services have been subsidised under the Australian Medicare Benefits Scheme since 1990 and associated pharmaceutical costs are also subsidised by the Pharmaceutical Benefits Scheme. Currently, fertility treatment services are broadly accessible as more than 50% of the direct treatment costs are covered under these schemes and there are no restrictions to access based on age, number of treatment cycles or existing family size (13, 14).

Linkage of fertility clinic data to the perinatal registry added information about diagnoses and conception with fertility treatment where the pregnancy was maintained for at least 20 weeks. Data linkage was performed using probabilistic matching software (Automatch V4.3, MatchWare Technologies) supplemented with manual checking and linking processes.

The study population was restricted to primiparous women in order to increase the likelihood that participants were employed in their designated usual occupation around the time of conception and to reduce potential bias associated with the 'infertile worker' effect (15, 16). This is an important consideration as half of Australian women (53%) reduce participation in the workforce after giving birth; while most return to work within two years, this is usually (84%) part-time, which would affect the exposure of interest in the study (17).

The study was approved by the ethics committees of the South Australian Department of Health, the University of Adelaide, and Flinders University. Individual patient consent was not required by the ethics committees.

*Night shift work*

The perinatal record includes a woman's usual occupation prior to and/or during pregnancy (usually transcribed from the first visit in the antenatal record); this data item appears at the beginning of the data collection form, before any details of medical history (18). Occupational data in the perinatal registry were recorded using the Australian Standard Classification of Occupations (ASCO) First Edition codes (19). To assess exposure to night shift work, a shift work job-exposure matrix (JEM) was applied to the occupational titles obtained from the perinatal registry for all primiparous women.

Job-exposure matrices provide a cross-classification of job titles and the probability of occupational exposure (20). Detailed description of the shift work JEM is published elsewhere (21). In a validation study, the JEM performed almost as well as job specific questionnaires in terms of reproducing an established association (21). To apply the JEM, International Standard Classification of Occupations (Revised Edition 1968) codes involving any exposure to shift work were translated into ASCO codes (19, 22). The JEM assigns each occupation a probability of exposure to light at night, phase shift, sleep disturbances, and other factors (23). For the present study, exposure to light at night was selected as a proxy for night and rotating shift work that includes nights. Occupations with exposure to light at night ("night shift occupations") were those in which at least 30% of workers reported exposure, an optimal threshold as determined in previous studies (24).

*Definition of variables*

In the first set of analyses, the outcome of interest was births to primiparous women who conceived by any form of clinic-based fertility treatment including in

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vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI), ovulation induction and intrauterine insemination. Births conceived to couples with male factor infertility as the primary infertility diagnosis were excluded from all analyses.

In the second set of analyses, the outcome of interest was infertility diagnosis among women who conceived with fertility treatment. There were six main categories of diagnosis: ovulatory dysfunction (including polycystic ovary syndrome), tubal blockage/problem, endometriosis (after visual inspection of the pelvic cavity), menstrual irregularity, and unexplained female-factor infertility (25). Menstrual irregularity was derived from recorded information about cycle length, either less than 24 days or greater than 32 days. Cycle length was self-reported by women at the beginning of each treatment cycle and includes those who reported an 'irregular' cycle rather than a cycle length in days. Apart from unexplained female-factor infertility, women could be assigned more than one diagnosis category.

Selected demographic, lifestyle, and health characteristics were obtained from the perinatal registry. Potential covariates considered included age ($<30$, 30-34, $\geq35$), ethnicity (Caucasian vs. non-Caucasian) and socioeconomic status determined using the postcode of residence and the Socio-Economic Indices for Areas (SEIFA) produced by the Australian Bureau of Statistics (26). Education level could not be considered as it is not routinely collected by the perinatal registry. Pre-pregnancy medical conditions considered in the analyses were diabetes, hypertension and asthma. Smoking status was routinely recorded on the perinatal record from 1998. Body mass index (BMI) was not routinely recorded during the study period (but was available for around three quarters of fertility treatment patients).

*Data analysis*

The proportions of primiparous women in a variety of occupations, classified according to night shift exposure, were examined. The proportions conceiving

with fertility treatment were calculated for these subgroups and for those not in the paid workforce (four subcategories).

Categorical variables describing health and sociodemographic profiles of women were tabulated, first according to night shift work status, then according to mode of conception. Corresponding chi-squared tests were undertaken to provide an initial guide as to the extent of confounding that might arise from these factors.

The association between night shift work and use of fertility treatment to conceive a first birth (binomial outcome) was investigated in detail using hierarchical logistic regression. A series of models were fit, beginning with an unadjusted model and progressively considering potential confounding factors. Covariates were retained if they met the following criteria: existing literature demonstrated or supported a plausible association with both shift work and infertility; the change in estimate (CIE) approach indicated the covariate was influential in model specification, determined by likelihood ratio testing (27).

BMI was not included in perinatal records in the study period so it could not be examined. Smoking was recorded for only part of the study period so sensitivity analyses were conducted using a restricted dataset containing this variable. Two reference groups were used. In the first instance, night shift working women were compared with all other women in paid employment who were not exposed to night shift. Secondly, comparison was made with a broader reference group that encompassed women who were not in the paid workforce.

For those primiparous women whose first birth was conceived with fertility treatment, infertility diagnoses were tabulated according to night shift exposure. Associations were investigated in detail using hierarchical logistic regression as above. Sensitivity analyses for smoking were undertaken as previously and additional sensitivity analyses for BMI were performed.

All hypothesis tests were two-sided and p-values < 0.05 were considered statistically significant. All data analysis was performed using Stata V.12. (StataCorp, College Station, Texas, USA).

**Results**

Of the 128,852 primiparous women who gave birth during the study period, a total of 11,000 (8.5%) were employed in occupations that were likely to have involved night shift (Table 1). The majority of night shift workers (72.7%) were registered or enrolled nurses (i.e. degree or diploma qualification). The largest occupational groups among their counterparts in paid employment but not exposed to night shift work were clerks and sales assistants, followed by teachers (excluding tertiary educators). One in five primiparous women were unemployed or engaged in home duties.

Overall, 1.6% of first births were conceived with fertility treatment (Table 1). For night shift workers the proportion was 2.2%. Use of fertility treatment for conception was least common among those unemployed, not in the labour force, or with unknown occupation. Births to women who were not in paid employment accounted for 14.6% of births from fertility treatment, compared to 25.9% of naturally conceived births.

**Table 1:** Births to primiparous women 1986–2002 by occupation and mode of conception.

| Employment status | All | | Proportion of night shift workers | Conceived with fertility treatment | |
|---|---|---|---|---|---|
| | N | % | % | N | % |
| All women | 128,852 | 100.0 | - | 2,058 | 1.6 |
| Night shift occupations | 11,000 | 8.5 | 100.0 | 243 | 2.2 |
| Registered nurses | 6,405 | 5.0 | 58.2 | 157 | 2.5 |
| Other personal service workers (e.g. croupier) | 1,818 | 1.4 | 16.5 | 32 | 1.8 |
| Enrolled nurses | 1,596 | 1.2 | 14.5 | 31 | 1.9 |
| Police | 383 | 0.3 | 3.5 | 11 | 2.9 |
| Radiographers | 209 | 0.2 | 1.9 | 5 | 2.4 |
| Food processing machine operators | 148 | 0.1 | 1.3 | 1 | 0.7 |
| Actors and related professionals | 103 | 0.1 | 0.9 | 0 | 0.0 |
| Misc. other shift working occupations[a] | 84 | 0.1 | 0.8 | 2 | 2.4 |
| Guards & security officers | 75 | 0.1 | 0.7 | 2 | 2.7 |
| Photographic products machine operators | 65 | 0.1 | 0.6 | 2 | 3.1 |
| Securities & finance dealers | 62 | 0.05 | 0.6 | 0 | 0.0 |

| | | | | | |
|---|---|---|---|---|---|
| Metal fitters & machinists | 52 | 0.04 | 0.5 | 0 | 0.0 |
| Selected non-night shift occupations | 84,991 | 66.0 | . | 1,514 | 1.8 |
| Other clerks | 13,071 | 10.1 | . | 248 | 1.9 |
| Sales assistants | 10,318 | 8.0 | . | 109 | 1.1 |
| Teachers[b] | 4,573 | 3.5 | . | 126 | 2.8 |
| Other or insufficiently described occupations | 1,869 | 1.5 | . | 21 | 1.1 |
| Not in paid employment | 32,861 | 25.5 | . | 301 | 0.9 |
| Home duties | 14,419 | 11.2 | . | 240 | 1.7 |
| Unemployed | 11,835 | 9.2 | . | 32 | 0.3 |
| Students | 3,416 | 2.7 | . | 14 | 0.4 |
| Pensioners | 477 | 0.4 | . | 3 | 0.6 |
| Unknown occupation | 2,714 | 2.1 | . | 12 | 0.4 |

a. Data combined for shift working occupations where n < 30 (air transport operating support workers, prison officers, production recording clerks, other stationary plant operators, fabric production machine operators).

b. Includes pre-primary, primary, secondary and extra-systematic teachers, but not tertiary teachers.

c. Couples who accessed fertility treatment for any diagnosis other than male factor infertility only.

As expected, maternal age, smoking, ethnicity and socioeconomic status were associated with conception using fertility treatment. Women in occupations involving night shift work tended to be older, Caucasian, and to live in the most economically advantaged areas compared to other employed women who were not exposed to night shift work (Table 2). Although there was a lower occurrence of smoking among night shift workers overall, smoking prevalence for occupations within night shift work was highly variable, for example, 4.9% for registered nurses, 12.2% for enrolled nurses and 26.7% for guards and security officers (data not shown). Socioeconomic status also varied within night shift working occupations; the proportion of women in the lowest socioeconomic quartile was 13.7% for registered nurses, 17.4% for enrolled nurses and 24.0% for guards and security officers. There was little difference in the overall prevalence of pre-pregnancy medical conditions among employed women when stratified by night shift work exposure.

The unadjusted analysis showed that primiparous women who worked night shift were 25% more likely to require fertility treatment to conceive a first birth (OR=1.25, 95% CI 1.09-1.43), compared to employed women who were not night shift workers (Table 3). This association changed marginally upon adjustment for ethnicity, socioeconomic status and asthma (with other medical conditions not meeting criteria for inclusion in the model). When age was added to the model, the association was attenuated (OR=1.10, 95% CI 0.95-1.26); indicating that women who worked night shift were older when they achieved their first birth, which could independently contribute to infertility but could also be a consequence of difficulty conceiving.

When the comparison group comprised all primiparous women (including those not in paid employment), the association between night shift and fertility treatment was stronger (OR=1.44, 95% CI 1.26-1.65). This result was somewhat attenuated upon adjustment for ethnicity, socioeconomic status and medical conditions. As above, including age in the model diminished the effect size (OR = 1.12, 95% CI 0.97–1.28).

**Table 2:** Demographic, health and lifestyle characteristics of primiparous women giving birth 1986–2002.

| Characteristic | Occupational groups | | | | | | | | Mode of conception | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Night shift workers (N=11,000) | | Non-night shift workers (N=84,991) | | Night shift vs non-night shift | Not in paid employment (N=32,861) | | | Fertility treatment (N=2,058) | | Natural (N=126,794) | | Treatment vs Natural |
| | N | % | N | % | P-value | N | % | | N | % | N | % | P-value |
| Age (years) | | | | | | | | | | | | | |
| < 30 | 7,139 | 64.9 | 60,185 | 70.8 | | 28,717 | 87.4 | | 579 | 28.1 | 95,462 | 75.3 | |
| 30–34 | 2,951 | 26.8 | 19,057 | 22.4 | < 0.001 | 3,059 | 9.3 | | 909 | 44.2 | 24,158 | 19.1 | < 0.001 |
| 35–39 | 797 | 7.3 | 5,027 | 5.9 | | 913 | 2.8 | | 474 | 23.0 | 6,263 | 4.9 | |
| ≥ 40 | 113 | 1.0 | 720 | 0.8 | | 169 | 0.5 | | 96 | 4.7 | 906 | 0.7 | |
| Smoking[a] | | | | | | | | | | | | | |
| Non-smoker | 3,561 | 79.8 | 28,906 | 76.0 | | 8,431 | 56.3 | | 1,512 | 82.3 | 39,386 | 70.8 | |
| Smoker | 877 | 19.6 | 8,855 | 23.3 | < 0.001 | 6,158 | 41.1 | | 324 | 17.6 | 15,556 | 28.0 | < 0.001 |
| Unknown | 26 | 0.6 | 283 | 0.7 | | 378 | 2.5 | | 1 | 0.1 | 686 | 1.2 | |
| Medical conditions | | | | | | | | | | | | | |
| Hypertension | 140 | 1.3 | 925 | 1.1 | 0.08 | 327 | 1.0 | | 28 | 1.4 | 1,364 | 1.1 | 0.2 |
| Epilepsy | 41 | 0.4 | 402 | 0.5 | 0.14 | 272 | 0.8 | | 13 | 0.6 | 702 | 0.6 | 0.6 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diabetes | 27 | 0.3 | 210 | 0.2 | 0.97 | 103 | 0.3 | | 6 | 0.3 | 334 | 0.3 | 0.8 |
| Asthma | 541 | 4.9 | 3,881 | 4.6 | 0.1 | 2,134 | 6.5 | | 82 | 4.0 | 6,474 | 5.1 | 0.02 |
| Ethnicity | | | | | | | | | | | | | |
| Caucasian | 10,716 | 97.4 | 81,581 | 96.0 | < 0.001 | 28,369 | 86.3 | | 1,978 | 96.1 | 118,688 | 93.6 | < 0.001 |
| Non-Caucasian | 284 | 2.6 | 3,410 | 4.0 | | 4,492 | 13.7 | | 80 | 3.9 | 8,106 | 6.4 | |
| Socioeconomic status | | | | | | | | | | | | | |
| Q1 (lowest quartile) | 1,708 | 15.5 | 17,114 | 20.1 | | 11,069 | 33.7 | | 350 | 17.0 | 29,541 | 23.3 | |
| Q2 | 2,386 | 21.7 | 21,010 | 24.7 | | 9,112 | 27.7 | | 428 | 20.8 | 32,080 | 25.3 | |
| Q3 | 3,012 | 27.4 | 21,165 | 24.9 | < 0.001 | 7,941 | 24.2 | | 493 | 24.0 | 31,625 | 24.9 | < 0.001 |
| Q4 (highest quartile) | 3,851 | 35.0 | 25,497 | 30.0 | | 4,625 | 14.1 | | 784 | 38.1 | 33,189 | 26.2 | |
| Missing | 43 | 0.4 | 205 | 0.2 | | 114 | 0.3 | | 3 | 0.2 | 359 | 0.3 | |

a. Routine reporting of maternal smoking on the perinatal record form did not begin until 1998. Therefore smoking data are unavailable for 71,377 pregnancies occurring before this date.

**Table 3:** Associations between night shift work and use of fertility treatment to conceive a first birth.

| | Use of fertility treatment | | Unadjusted model | Adjusted model | Adjusted model + age |
|---|---|---|---|---|---|
| | Night shift workers N (%) | Reference group N (%) | OR (95% CI) | OR (95% CI) | OR (95% CI) |
| Night shift workers[a] vs non-shift employed workers[b] | 243 (2.2) | 1,514 (1.8) | 1.25 (1.09–1.43) | 1.20 (1.05–1.38) | 1.10 (0.95–1.26) |
| Night shift workers[a] vs all other women[c] | 243 (2.2) | 1,815 (1.5) | 1.44 (1.26–1.65) | 1.33 (1.16–1.53) | 1.12 (0.97–1.28) |

a: N = 11,000; b: N = 84,989; c: N = 117,852

CI: confidence interval; OR: odds ratio

In the period in which information on smoking was collected routinely in the perinatal record (1998-2002), smokers were 60% less likely to have conceived using fertility treatment (consistent with finding for socioeconomic status). Inclusion of smoking in the fully adjusted model did not alter the overall association between night shift work and use of fertility treatment for conception, regardless of the comparison group (result not shown).

The diagnoses among women who received treatment for infertility were then examined by night shift work exposure. Table 4 shows the prevalence of key conditions contributing to infertility for the following groups of primiparous women: night shift workers, employed women who did not work night shift, and all women other than night shift workers.

Endometriosis and menstrual irregularity were more common among night shift workers compared to other employed women who did not work night shift or to all other women (Table 4). In contrast, there was little difference in the prevalence of unexplained infertility, ovulatory dysfunction and tubal problems among groups of women.

In sensitivity analyses, data were restricted to the period in which smoking was routinely recorded. Including smoking status in the models did not appreciably change the effect estimates presented in Table 4 for ovulatory infertility and menstrual irregularity. For endometriosis and tubal infertility the effect estimates increased (protective direction for the latter), while for unexplained infertility the odds ratio weakened. These changes are consistent with smoking being associated with these three outcomes and more common amongst non-shift workers. Including BMI in the models, where available, made little difference to the magnitude of any associations. Choice of reference group (women in paid employment or all other women) did not affect these results.

**Table 4:** Associations between female infertility categories and night shift work among women who required fertility treatment to conceive a first birth.

| | Night shift workers (N=243) | | Non-night shift workers (N=1,514) | | Night shift vs non-night shift workers | | | | All other women (including not in paid employment) (N=1,815) | | Night shift workers vs all other women | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Unadjusted | | Adjusted[a] | | | | Unadjusted | | Adjusted[a] | |
| | N | % | N | % | OR | 95% CI | OR | 95% CI | N | % | OR | 95% CI | OR | 95% CI |
| Ovulatory dysfunction | 48 | 19.8 | 318 | 21.0 | 0.93 | 0.66-1.30 | 0.90 | 0.64-1.27 | 379 | 20.9 | 0.93 | 0.67-1.30 | 0.93 | 0.66-1.31 |
| Endometriosis | 76 | 31.3 | 390 | 25.8 | 1.31 | 0.98-1.76 | 1.34 | 1.00-1.80 | 451 | 24.8 | 1.37 | 1.03-1.84 | 1.39 | 1.04-1.87 |
| Tubal blockage/problem | 77 | 31.7 | 520 | 34.3 | 0.89 | 0.66-1.19 | 0.88 | 0.65-1.18 | 648 | 35.7 | 0.84 | 0.63-1.11 | 0.82 | 0.62-1.10 |
| Menstrual irregularity | 76 | 31.3 | 36 | 24.2 | 1.42 | 1.06-1.91 | 1.42 | 1.05-1.91 | 451 | 24.8 | 1.38 | 1.03-1.84 | 1.38 | 1.03-1.85 |
| Unexplained infertility | 31 | 12.8 | 269 | 17.8 | 0.68 | 0.45-1.01 | 0.69 | 0.46-1.03 | 307 | 16.9 | 0.72 | 0.48-1.07 | 0.73 | 0.49-1.08 |

a. Adjusted for age, ethnicity, socioeconomic status.

CI: confidence interval; OR: odds ratio

**Discussion**

We found that, compared to women in paid employment who did not undertake night shift, women who worked night shift as per a job-exposure matrix were between 10% and 25% more likely to conceive their first birth using fertility treatment. To our knowledge this is the first study to investigate night shift work and use of fertility treatment. Our results are consistent with a Danish study which investigated industrial differences in female fertility treatment rates, finding that hospital workers – among whom night shift is common - were significantly more likely to undergo fertility treatment (28).

As infertility entails a longer time to become pregnant, it follows that night shift workers would be somewhat older than their counterparts at the time of conception. In this way, age is not entirely an independent contributing factor. In addition, night shift work may contribute to a delay in family formation through social pathways reflecting life course decisions made by highly educated women or impacts of shift work on relationships and intimacy (3, 29). Our findings about infertility diagnosis shed some light on this, with specific diagnoses elevated among night shift workers who conceived with fertility treatment, but no difference in unexplained infertility, pointing more strongly to age as part of the causal web. Thus, the main finding should not be viewed simply as a consequence of older age when attempting motherhood.

In particular, night shift workers who received fertility treatment were more likely to have an infertility diagnosis of endometriosis or menstrual irregularity compared to other women requiring treatment to achieve a first birth. These associations are consistent with previous literature on shift work, menstrual irregularity and long time to pregnancy (8, 11), and a much smaller literature on shift work and endometriosis (9, 10).

The more frequent diagnoses of menstrual irregularity and endometriosis among those night shift workers requiring fertility treatment are consistent with biological mechanisms associated with night and rotating shift work. Different hormone systems follow different secretory patterns and adapt at different rates to

circadian disruption, so night and rotating shift work is likely to produce at least some asynchrony in these systems (5, 30). Animal studies suggest that optimal functioning of the suprachiasmatic nucleus is required to produce the luteinizing hormone (LH) surge and ensuing ovulation and that melatonin interacts with gonadotropins, including augmentation of the LH surge (5, 31). In this circumstance, perturbation of the LH surge may disrupt the cyclicity of ovulation in women who otherwise do not have anovulatory infertility or poor ovarian reserve.

Circadian misalignment and impaired sleep are also associated with neuroendocrine stress (increased cortisol and catecholamine activity), oxidative stress, altered immune function and low-grade system inflammation (32). Impaired immune function and inflammatory responses in night shift workers may contribute to increased susceptibility to endometriosis, as impaired immune surveillance and reactive oxygen species have been implicated in the inflammatory and pathophysiological processes of the disease (33-35).

Individuals have been shown to vary in their ability to tolerate night shift work. Those who do not tolerate night shift experience symptoms associated with circadian disruption such as gastrointestinal disturbance, sleep disturbance, fatigue and changes in mood (irritability, low affect) and behaviour (36, 37). Thus, there is likely to be some self-selection into or out of undesired shift schedules (38). It is possible night shift workers who required fertility treatment for a first birth had relatively poor tolerance for shift work, but limited choice about the matter.

Three quarters of women exposed to night shift work were nurses. As such, the majority had considerable knowledge of health and the health care system, so may have been more accepting of medical intervention for infertility. There were too few women exposed to night shift work in other industries, of similar socio-economic status, to undertake sensitivity analyses to determine the role of the employment sector. However, the specific findings on diagnoses add support to the biological effects of night shift work. In addition, an association between shift work and menstrual irregularity has been demonstrated in studies of different design. This includes questionnaire-based studies, where collection of outcome

data was not associated with accessing clinical treatment for infertility (8), and studies where nurses did not form the majority of the sample (39).

Apart from age and smoking, the covariates considered had little influence on findings. Smoking was more prevalent among women who did not work night shift. Since smoking is also weakly protective for endometriosis (40), positively associated with risky sexual behaviours (hence tubal infertility) (41), and positively associated with recurrent very early miscarriage (hence unexplained infertility) (42, 43), adjusted effects are in expected directions, but do not change the key findings.

The elevated use of fertility treatment among night shift workers was magnified when the comparison group comprised all primiparous women, including those not in paid employment. The group of women engaged in home duties was larger than expected for primiparous women; the great majority of these women had their first birth at less than age 30 years and were relatively disadvantaged, (suggesting weaker ties to career and jobs lacking paid maternity leave). Since usual occupation is reported at the time of birth, when women are no longer in paid employment, a degree of non-reporting of former occupation is likely among such women (18). It is difficult to gauge whether misclassification bias could arise from this source, but some reassurance is provided by the fact that assisted conception occurred in 1.7% of women reporting home duties, similar to the proportion for women in paid employment who did not work night shift (1.8%).

Strengths of this study are the large, population-based cohort of over 128,000 primiparous women, and the detailed health information available for women undergoing fertility treatment. Restriction of the analysis to primiparous women substantially addresses any bias due to the infertile worker effect, whereby childless women are more likely to remain in the workforce (15). The job-exposure matrix (JEM) used was developed in a representative population of women of the same nationality and contemporary to the study population. JEMs are a well-accepted and commonly-used method to extrapolate exposure from occupational data where direct measurements cannot be made (44, 45). A further

strength of a JEM is that it is applied consistently to all study participants, attenuating observation bias or at least rendering it non-differential.

The use of JEMs to classify exposure also has some limitations. JEMs classify exposure at the occupation-level rather than the individual-level. There is therefore likely to be some misclassification of exposure. However, as misclassification occurs independently of outcome status, i.e. non-differentially, this tends to lower risk estimates. We were also unable to consider other hazardous exposures that may affect reproductive health. Nurses, for example, are potentially exposed to a number of hazards including antineoplastic drugs, solvents and physically demanding work (8, 46), the effects of which could not be separated from shift work.

It is also important to note that women who access fertility treatment may not be representative of all infertile or subfertile women, particularly in terms of socioeconomic status. Although socioeconomic status was adjusted for in the analyses, it cannot address potential selection bias associated with the construction of the sample. Given the modest effect sizes observed in this study, it is possible that unrecognised bias or residual confounding may play a role. A further limitation of this study is that we do not have information on fertility treatment where it was sought but a woman either did not conceive or gestation did not reach 20 weeks. While difficult to ascertain due to clinics focussing on outcomes per cycle, it is estimated that this applied to around half of women who attended fertility clinics at this time (47). We also do not have information on menstrual irregularity or endometriosis among women who conceived naturally.

In conclusion, this study adds to literature implicating night shift work in reproductive health problems (3, 8, 11). Adverse effects may be most pronounced in a vulnerable subgroup with poor tolerance of the sequelae of night shift work and this deserves further research. Providing these women with a degree of control and choice about shift schedule may be the best way to enable them to maintain income and career and health, while accommodating shift work (48). Other strategies to mitigate circadian disruption exist, for example tailored sleep plans (49); these should be promoted and further practical avenues explored.

**Authors' contributions:**

R.C.F. participated in study design, planned and conducted the statistical analysis, interpreted the results and drafted the manuscript. V.M.M, J.L.M and M.J.D participated in study design, interpretation of results, and critically reviewed and contributed to the manuscript.

**Conflict of interest:**

Authors declare no conflicts of interest.

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# 6 Project Four: Maternal night shift work, ART and urogenital anomalies among first births

## 6.1 Introduction

Project four describes a series of analyses that aim to identify, first, whether maternal night shift work is a patient factor contributing to increased risk of urogenital anomalies among births conceived with fertility treatment and, secondly, how patient and treatment factors may be distinguished. The section begins by outlining the rationale for this investigation and additional methodological considerations relevant to this project. This is followed by the manuscript presenting the study.

**Rationale**

*Why urogenital anomalies*

It is now widely known now that congenital anomalies occur more frequently among children conceived with medical assistance compared to their naturally conceived counterparts.[7, 74] This extends to urogenital anomalies, which are among the most commonly diagnosed congenital anomalies, affecting up to 16.7 per 1,000 births, with significantly higher prevalence of 23.5 per 1,000 among births conceived using ART.[7, 59]

There are now mechanistic arguments and preliminary empirical evidence to suggest that specific types of anomalies may be increased or decreased by specific elements of the fertility treatment process, which may have effects on different target tissues or systems and/or act at different stages of early development. For example, Tamoxifen is a drug that can be used to treat anovulatory infertility.[176] It is classed as a selective estrogen receptor modulator (SERM), which has tissue-specific estrogen antagonist and agonist effects.[177] Due to its long half-life, Tamoxifen remains detectable for several weeks, meaning that the developing fetus is potentially exposed should pregnancy occur. It has been noted that

SERMs interact with rapidly growing and developing embryonic or fetal tissues.[177] Disruption of angiogenesis by SERMs has potentially catastrophic consequences for the developing embryo and fetus.

In addition, there are plausible mechanisms linking circadian rhythms to perturbed maternal endocrinology and subsequent fetal exposure to hormonal disturbances in utero. For example, altered endocrinology are observed consequences of circadian disruption,[109] and there is evidence that the aetiology of some types of urogenital anomalies, such as hypospadias, may be influenced by hormonal balance in utero.[8]

### *Legacy of urogenital anomalies*

Congenital anomalies, including urogenital anomalies, have an enduring legacy that can affect the child's physical, mental and emotional quality life into adulthood. Hypospadias, where the urethral opening is on the underside or shaft of the penis instead of the tip, is a commonly occurring urogenital anomaly affecting 3.8 per 1,000 male births.[59] It is most often treated with surgical repair in early life. Despite corrective treatment, studies of adult men who underwent the procedure in infancy report higher rates of urinary symptoms and psychosocial stress relating to cosmetic appearance and sexual function, including erectile and ejaculatory dysfunction.[178] Hypospadias often co-occurs with undescended testes. Undescended testis is another common urogenital anomaly that is linked impaired fertility and increased risk of malignancy in affected men, particularly if corrective surgery is delayed beyond 12 months of age.[179] Even among men who underwent timely corrective surgery, there is evidence to suggest that semen quality is impaired.[180]

Renal and urinary anomalies occur less frequently, thus little is known about their aetiology and long term outcomes. Examples of these anomalies include renal agenesis or dysgenesis and vesicoureteral reflux.[181] Renal and urinary anomalies account for approximately 50% of chronic kidney disease in childhood and early adulthood. They are also a major contributor to end stage renal disease and the need for renal replacement therapy, which is associated with cardiovascular

morbidity, impaired growth, psychosocial problems and increased mortality in children.[182]

*Evidence for the role of patient factors*

When investigating the outcomes of fertility treatment, it is important to consider both patient and treatment factors. As described earlier in this thesis, the evidence informing this approach comes from observations of increased risk of congenital anomalies among babies conceived spontaneously by couples with a long time to pregnancy.[74]

There is preliminary evidence that night shift work influences female reproductive systems and fertility,[183] which provides grounds for considering maternal night shift work as a risk factor for urogenital anomalies in offspring. The research questions guiding the fourth project of this thesis are:

1. Does the distribution of fertility treatment types vary between night shift workers and non-shift workers?
2. Does maternal night shift work increase the risk of urogenital anomalies and does this relationship vary according to the presence or absence of conception using fertility treatment?
3. Is the risk of urogenital anomalies greater for night shift workers who undergo fertility treatment compared to non-shift workers who undergo fertility treatment?

## 6.2 Additional methodological considerations for project four

Reproductive and perinatal epidemiological involves some special methodological issues that must be considered in addition to the general concerns of study design, power and bias. This section will discuss some of these issues in more detail and explain the approaches taken to mitigate these issues in this project.

**Clustering of outcomes**

Clustering occurs when individual observations in a study are grouped in some way. There may be some common factor between two or more observations, which means that they cannot be treated as independent observations, hence, violating the requirements of commonly applied statistical analysis techniques.[184] Clustered data may occur in multi-centre randomised controlled trials, cohort studies with repeated measures or when data are collected at several levels, e.g. individual, family, community.[185]

In reproductive and perinatal epidemiology there are two common sources of clustering. The first and most obvious source of clustering occurs in the case of multiple gestation.[186] Babies who are twins, triplets or higher order multiples share among other things, genes, the uterine environment and maternal conditions during pregnancy. The second source of clustering is serial pregnancies to the same woman.[186] This is likely to occur in longitudinal cohort studies, particularly those involving administrative data, such as birth registries. Data analysis in perinatal studies can be further complicated by the presence of both independent (singleton births to different women) and clustered outcomes in the one dataset.[184]

Clustering within a cohort means that there is less information than would be the case if all observations were truly independent. The effective sample size is reduced in clustered data, leading to increased uncertainty in the results of statistical analyses.[186] Ignoring the presence of clustering when conducting statistical analysis can produce incorrect estimates of variance and statistically inefficient estimates of regression parameters.[187]

Straightforward, but not necessarily epidemiologically sound, methods for dealing with clustering include limiting analysis to first born singletons from each family or using data from one of a multiple birth. Both of these methods are inefficient in terms of making use of all the available data.[188]

There are several statistical techniques that can be used to take account of clustering however, historically, these have not often been used in practice. In

2010, Hibbs et al. conducted a systematic review looking at how multiple births were accounted for in perinatal and neonatal trials.[184] Only four out of 41 studies used statistical techniques that accounted for clustering. These four techniques were data management approaches such as excluding multiples or including one baby from a multiple birth, a statistical technique (generalised estimating equations, GEE), randomisation of the mother in prenatal interventions and stratified randomisation of multiples and singletons. After applying each of these techniques to a case study, Hibbs et al.[184] concluded that statistical methods (e.g. GEE) that account for clustering produced the most valid point estimates of effect size and most conservative confidence interval estimates.

The statistical approaches used to account for clustering can be classified under two main categories, conditional and marginal approaches.[186] Conditional approaches, such as hierarchical, multilevel modelling or random effect modelling, produce cluster-specific effect estimates. Marginal approaches, such as GEE methods, produce effect estimates that are interpreted at a population-average level. This distinction in the interpretation of the estimates has important implications for the choice methods. The use of conditional methods conditions on cluster size and treats singletons and multiples as the same. This is not appropriate in cases where the data contain clustering from both multiplicity and serial pregnancies to the same women, as multiplicity could be on the causal pathway. Therefore, the GEE method has been applied in all analyses in this thesis that involved clustered data.

### Critical windows

The 'when' or timing of exposure is particularly important for studies of outcomes relating to human reproduction. Human reproductive and developmental processes are highly regulated and inter-related, and in many cases there are specific critical windows in which exposure must occur in order to interrupt reproductive or developmental capacities.[186] Adding further complexity is the spread of critical windows across the life span, which has led to greater interest in life course and intergenerational health research.[189]

Intrauterine programming refers to a 'process whereby a stimulus or insult, at a critical period of development, has lasting or lifelong significance' (Barker 1994).[190(p14)] Depending on the type of insult, and the timing, duration and severity of exposure, changes in the intrauterine availability of nutrients, oxygen and hormones that program tissue development and growth can occur. The mechanisms of intrauterine programming include structural and/or functional changes in genes, cells, tissues of whole organs. In addition, the same exposure at different times (or at different doses or intensities) may produce different effects. For example, exposure around the time of conception and during early pregnancy may produce spontaneous pregnancy loss, whereas exposure later in pregnancy may produce congenital anomalies or impair tissue function and growth that leads to cardiovascular and metabolic abnormalities in the child or that manifest as adult disease. The five critical periods of intrauterine programming described by Fowden et al.[191] are summarised in Figure 5 below.

Figure 6 summarises the critical windows that are relevant specifically for congenital anomalies. During the first eight weeks of gestation the fetus is highly susceptible to morphological abnormalities. This pattern of sensitivity reflects the timing of organ system development in utero.[192] Exposure after this time may lead to minor morphological abnormalities or changes in function or growth.[193, 194] Major congenital anomalies are most likely to occur when exposure to teratogens occurs early in pregnancy, i.e. during organogenesis phase. However, some authors extend the term teratogenicity to include not only structural malformation but functional impairment, impaired viability and growth restriction.[193]

**Figure 5:** Five critical periods of intrauterine programming. Adapted from Fowden et al.[191]



| Periconceptual / preimplantation | Placentation phase | Organogenesis phase | Rapid fetal growth phase | Late gestation / fetal maturation |
|---|---|---|---|---|
| • Nutrient, oxygen and hormone levels affect oocyte and blastocyst development<br>• Methylation patterns | • Environmental programming mediated by changes in placental development | • Structural defects that produce permanent functional impairment | • Availability and utilisation of nutrients impacts tissue growth | • Alterations in physiological systems that are activated perinatally |

**Figure 6:** Critical periods for congenital anomalies, based on the timing of organ system development. Adapted from Moore et al.[192], Selevan et al.[194].
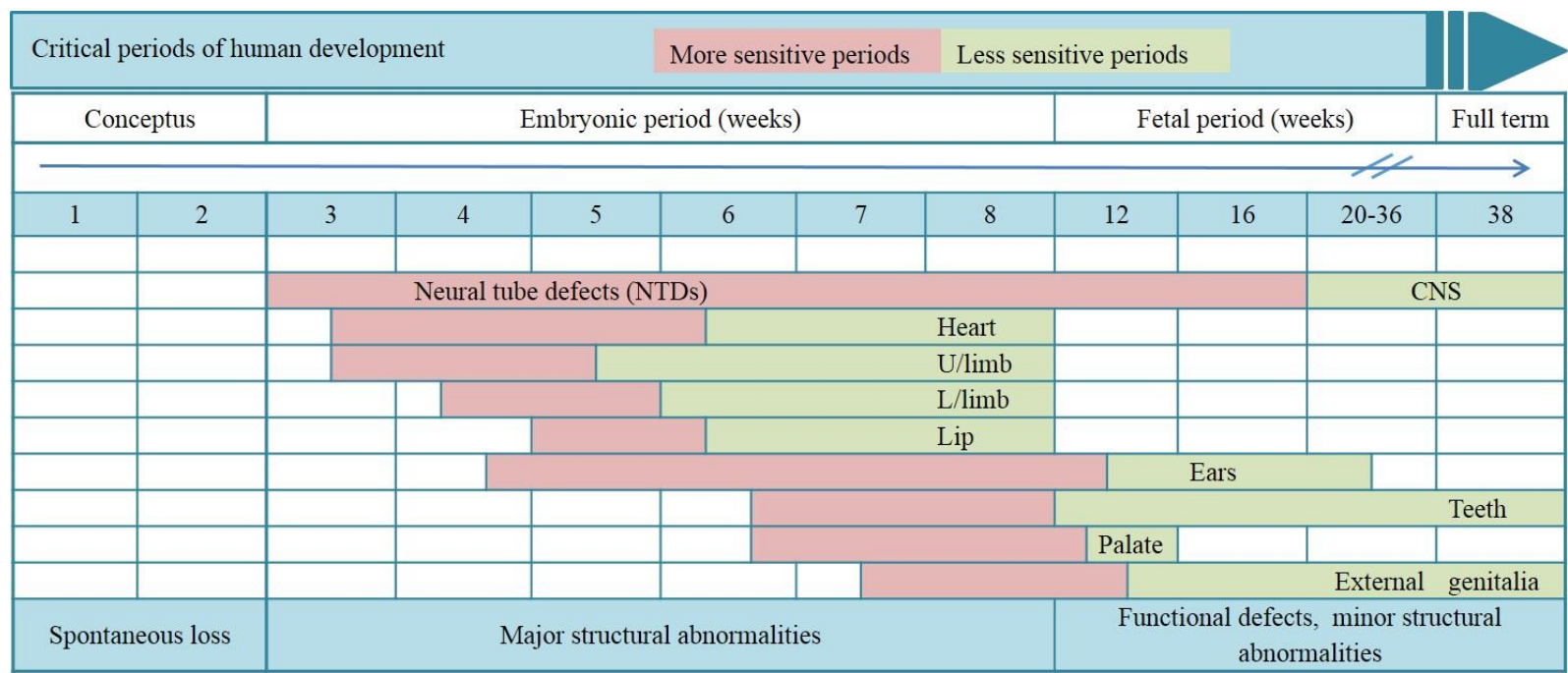
Ionizing radiation is one example of an environmental teratogen, where a prenatal critical window and threshold exposure level has been established. The period of susceptibility is 8–15 weeks gestation and the threshold dose 20 rad (although 5 rad is used when counselling pregnant women). Potential teratogenic effects include mental retardation and microcephaly.[193]

Critical windows are important in determining the contribution of an environmental exposure to pregnancy and child health outcomes, but are often inadequately addressed in the design of epidemiological studies. In cases of prenatal exposure, exposure patterns are often determined retrospectively and involve reconstruction or recall by study participant. Establishing the time frame of exposure is therefore subject to recall error and potentially recall bias (in case-control studies).[194] Similarly, in studies of postnatal exposures, if assessment of exposure only occurs at the time that the child is diagnosed with a condition, this may not allow accurate ascertainment of the critical period of exposure.[194]

The critical time periods of interest to the present study are the preconception and prenatal periods. In an effort to target these periods more specifically, analyses were limited to the first pregnancy for each woman. It improves the likelihood that the woman was actually working in the occupation reported as her usual occupation at the time of conception. This is because many women having a second (or later) birth, may actually be working part-time or not in the paid workforce, so they can care for their firstborn.[174]

**Bayesian statistics to overcome problems with sparse data**

Issues relating to sparse data can occur in studies of small populations, rare outcomes or short follow up times. Sparse data can prohibit model convergence, or if convergence is achieved, produce unstable effect estimates that lack precision.[195] Bayesian statistical methods are one way to overcome problems with the analysis of sparse data and these methods are considered by some to be a better choice when samples are generated in a non-random way, which is the case in many epidemiological studies.[196]

In short, Bayesian analysis incorporates prior knowledge about the relationships between the parameters under investigation with the actual study data to produce posterior probabilities, which can be used to make inferences.[197] Bayesian analysis can be conducted using a data augmentation approach, or the more computationally intensive Markov Chain Monte Carlo (MCMC) method.[142]

The data augmentation approach involves the incorporation of prior information by adding observations to the dataset. This can then be analysed using standard statistical software and regression analysis techniques to produce posterior effect measures (e.g. odds ratios) and posterior intervals.[198] Briefly, the Markov Chain Monte Carlo (MCMC) is a simulation based method whereby Markov chains are used to iteratively generate samples from posterior distribution of the model parameters and Monte Carlo integration is used to produce summary estimates from the samples.[199, 200] This requires extensive computational resources. For the present project, the data augmentation approach was chosen over MCMC, as it is easier to implement, is less computationally intensive, less prone to technical problems and can be performed using standard statistical software packages, which until recently was not the case for MCMC.[142, 196]

In the data augmentation approach, observations are added to the real dataset to stabilise covariates and to ensure that the prior and data models are adequately approximated by the normal distribution.[196] One data record is added for each covariate. A stable covariate is one in which there are least five cases and non-cases.[201] For covariates that are already stable before the addition of prior data, researchers may choose not to add a prior (semi-Bayesian analysis) or to add a very weak prior that is unlikely to have a large effect on the results (full Bayesian analysis).[142]

The specification of the prior distribution, representing prior knowledge about the variables in the model, may be informative (weakly to strongly) or non-informative.[197] Weakly informative priors serve to stabilise effect estimates, which are pulled towards the null, without having a large influence on the size of

posterior effect estimates.[202] An example of weakly informative priors would be a prior distribution where the odds ratio is equal to one.

Informative priors may be used when the relationships between covariates are well known from previous research. For example, it is well known that the risk of birth defect increases with maternal age. The specification of informative priors can be controversial, as the choice of inappropriate priors can lead to inappropriate conclusions.[200] One way around this would be to conduct sensitivity analyses in which the prior probably distribution is varied.[200]

For the present analysis, a fully Bayesian approach was used, whereby weakly informative priors were chosen for all variables in the model. The weakly informative priors were null centred, with odds ratios equal to one and standard error approximately equal to two. Appendix 3 outlines the steps involved in Bayesian data augmentation using null priors in practical terms.

**Analysis of interaction effects**

Formal testing of interaction effects is usually performed by adding an interaction term (i.e. variable 1 x variable 2) to a statistical model. This approach provides a clear indication as to whether an interaction effect is present, however, interpretation of output from such a model is not straightforward, particularly on the odds ratio scale, as when logistic regression is performed.[203] In order to present the interaction results from project four in a more accessible fashion, the relevant literature was consulted for advice on how to perform, present and interpret an interaction analysis.

The results of the interaction analysis are presented in the following manuscript according to the recommendations of Knol & VanderWeele.[203] In order to provide enough information such that reader can easily gauge the size and statistical significance of interactions, these authors recommend that the separate effects of each independent variable (night shift work, ART) are presented, along with the combined effects of these variables. These should be compared to a single reference category, usually the group with the lowest risk of the outcome of

interest (urogenital anomalies), for example, in the present study this is the naturally conceived group with no maternal night shift exposure.

The results within strata of each independent variable should be presented, together with measures of interaction on either the additive or multiplicative scale (with their confidence intervals and p-values), or both.[203] An example of a measure of interaction (between two variables A and B) on the additive scale is the relative excess risk due to interaction (RERI), which is calculated using the formula: $OR_{11} - OR_{10} - OR_{01} + 1$.[204] Where $OR_{11}$ is the odds ratio for the interaction term (i.e. both A and B = 1), $OR_{10}$ is the odds ratio for A (where B is set to 0) and $OR_{01}$ is the odds ratio for B (where A is set to 0). This value indicates whether the effect of both exposures combined is greater than the sum of the two exposures considered separately. An additive interaction is positive if RERI $>0$ and negative if RERI $<0$. The RERI provides the direction, but not the magnitude, of an additive interaction.[205] A measure of interaction on the multiplicative scale is the ratio of odds ratios, $OR_{11} / OR_{10} * OR_{01}$.[205] This value indicates whether the effect of both exposures combined is greater than the product of the two exposures considered separately. A positive multiplicative interaction is indicated by $OR_{11} / OR_{10} * OR_{01} > 1$ and a negative multiplicative interaction by $OR_{11} / OR_{10} * OR_{01} < 1$.[205] In the above equations, relative risk terms have been replaced with odds ratios. In the present analysis, odds ratios are assumed to approximate relative risk, as birth defect outcomes are relatively rare.[206]

Although it is good practice to provide both additive and multiplicative measures of interaction, an additive interaction may be considered more relevant from a public health perspective.[203, 205] This is because it can be used to identify population subgroups that are more susceptible to an exposure, or alternatively, would benefit more from an intervention or treatment.

## 6.3 Statement of authorship

# Statement of Authorship

| Title of Paper | Is maternal shift work contributing to the excess of urogenital defects after infertility treatment? |
|---|---|
| Publication Status | ☐ Published      ☐ Accepted for Publication <br> ☐ Submitted for Publication    ☑ Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | - |

## Principal Author

| Name of Principal Author (Candidate) | Renae Clare Fernandez |
|---|---|
| Contribution to the Paper | Contributed to study conception and design. Assisted with cleaning of data for analysis. Planned and conducted the statistical analysis. Interpretation of results. Drafted the manuscript and acted as corresponding author. |
| Overall percentage (%) | 80% |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |
| Signature |     | Date | 14/12/2016 |

## Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

    I.    the candidate's stated contribution to the publication is accurate (as detailed above);

    II.    permission is granted for the candidate in include the publication in the thesis; and

    III.    the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

| Name of Co-Author | Vivienne M. Moore |
|---|---|
| Contribution to the Paper | Contributed to conception and design of the study. Supervised the analysis and interpretation of research data. Critically revised the manuscript. |
| Signature |     | Date | 14/12/2016 |

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|---|---|---|---|
| Contribution to the Paper | Contributed to conception and design of the study. Extracted and cleaned the data for analysis. Supported the statistical analysis and provided advice on Bayesian data augmentation. Contributed to interpretation of the findings. | | |
| Signature | | Date | 18/01/2017 |

| Name of Co-Author | Michael J. Davies | | |
|---|---|---|---|
| Contribution to the Paper | Led conception and design of the study. Critically revised the manuscript. | | |
| Signature | | Date | 14/12/2016 |

Please cut and paste additional co-author panels here as required.

## 6.4 Manuscript

**Title**: Does maternal shift work contribute to the excess of urogenital defects after fertility treatment?

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## Abstract

**Background:** Urogenital anomalies are more common among births conceived with fertility treatment. This may reflect patient factors that underlie infertility or treatment effects. It is unknown whether maternal night shift work is a patient factor that contributes to the risk of urogenital anomalies in babies conceived with fertility treatment.

**Methods:** A population-based cohort was produced via data linkage. A job-exposure matrix was applied to usual occupation to impute maternal night shift exposure. The joint effects of maternal night shift work and mode of conception on urogenital anomalies in first births were examined using logistic regression, including an interaction term, while adjusting for potential confounders. Among births from fertility treatment, associations between treatment type and maternal night shift exposure were examined.

**Results:** Among multiple first births conceived with fertility treatment, the risk of urogenital anomalies was significantly higher for births to night shift workers compared to non-shift workers (aOR = 2.94, 95% CI 1.26 -6.85). The risk of urogenital anomalies was elevated, but did not reach statistical significance for single first births conceived with fertility treatment (aOR = 1.80, 95% CI 0.94-3.46). This was not related to differences in the type of treatment received by night shift workers compared to non-shift workers. Among first births conceived naturally, maternal night shift work was not associated with risk of urogenital anomalies in singletons (aOR=1.01, 95% CI 0.86-1.18) or multiples (aOR = 0.54, 95% CI 0.11-2.70). A statistically significant additive interaction indicated that the combined effects of maternal night shift work and fertility treatment were more detrimental than either exposure in isolation. There was also some evidence of a positive multiplicative interaction, but this was not statistically significant.

**Conclusions:** In a subgroup of women, night shift work may affect reproductive health, inducing subfertility. When these women conceive with fertility treatment, offspring have heightened risk of urogenital anomalies due to both patient and treatment factors.

## Introduction

Urogenital anomalies are among the most commonly diagnosed congenital anomalies, affecting up to 16 per 1,000 births per year, with significantly higher prevalence among births conceived using assisted reproductive technologies.[1, 2] There is evidence that the higher risk of congenital anomalies overall among births from fertility treatment is related to both treatment factors, such as invasiveness of treatment, and patient factors, including the severity of infertility.[3] A role for patient factors is supported by an observed increased risk of congenital anomalies among subfertile couples (i.e. with a prolonged time to pregnancy) who conceived naturally.[4]

It is plausible that patient factors that underlie infertility could contribute to complications in pregnancy and impaired embryonic and fetal development.[5]

Often genetic factors are raised, but exogenous factors such as maternal occupational exposures could be involved.

Night and rotating shift work usually involves exposure to light at night. Exposure to light at night is known to interfere with circadian rhythms, which are co-ordinated by the suprachiasmatic nucleus in the hypothalamus.[6] The suprachiasmatic nucleus is responsible for relaying circadian information to other central and peripheral circadian oscillators via the regulation of clock-gene expression and neuroendocrine signalling,[7, 8] such as through the rhythmic secretion of melatonin by the pineal gland.[9]

Circadian clock-gene expression has been observed in several reproductive tissues including the ovary, which may explain why alterations in endogenous levels of other hormones, including estrogen, have also been observed among shift workers.[10, 11] Circadian disruption of melatonin secretion is also implicated in reproductive function, particularly during pregnancy, as it has been shown to be important in regulating the fetal circadian rhythm and as an antioxidant.[12]

The development of the male urogenital system in utero occurs in a hormone-dependent manner; therefore exposures that disturb the endocrine system, such as night shift work are potentially implicated in the development of urogenital anomalies.[13] However, no previous studies have investigated the occurrence of urogenital anomalies specifically among infants born to mothers who work night shift.

Aside from the effects of night shift work on the endocrine system, there is also evidence that night shift work produces or exacerbates fertility problems, and therefore recourse to treatment. Studies have observed higher rates of menstrual disturbance, endometriosis and miscarriage among shift workers.[14-16] We have found higher uptake of fertility treatment by women in occupations involving night shift work (Fernandez et al. 2017, submitted).

Using a population-based cohort, the present study aims to establish whether maternal night shift work is a patient factor that contributes to the increased risk

of urogenital anomalies among births conceived using fertility treatment, and to determine whether this is related to differences in treatment types in this group.

**Methods**

*Data sources and study population*

The study population comprised all live births, stillbirths, and terminations for defects after 20 weeks occurring among women residing in South Australia (SA) between 1986 and 2002. The study cohort was produced by linking data from three sources, including two routine data collection registries as described in detail previously.[1]

By law, all live births and stillbirths (of at least 20 weeks' gestation or with a birth weight of at least 400 g) and all medical terminations for defect occurring after 20 weeks gestation are reported to the State Perinatal Statistics Collection. This registry also collects information on maternal demographics, including usual occupation, pre-existing medical conditions, and medical complications during pregnancy. Smoking status was routinely recorded on the perinatal record from 1998. Body mass index (BMI) was not routinely recorded during the study period (but was available for around three quarters of fertility treatment patients).

Data on the outcome of interest, urogenital anomalies, was obtained from the South Australian Birth Defects Register. Congenital anomalies are reportable until a child's fifth birthday, thus are not limited to those readily detected in the neonatal period. Structural, biochemical, chromosomal and other genetic anomalies are included and classified according to the British Paediatric Association Modification of the International Classification of Diseases 9th Revision. All codes relating to urogenital anomalies, ICD-9 BPA 75200 – ICD-9 BPA 75399 were included in the study. Minor anomalies, for example hydrocoele testis, are excluded from the register unless they are disfiguring or require treatment.

Data relating to births conceived using fertility treatment was obtained from the two clinics that were registered at the time to provide treatment involving manipulation of gametes and embryos in SA. This also included data on patients who received less invasive treatment within the clinic setting, including treatment with ovulation induction drugs only. Women were excluded if they were missing an infertility diagnosis (n=12 or 0.01%).

The SA Department of Health performed the data linkage. A unique accession number was used to link State Perinatal Statistics Collection data to the Birth Defects Register. Linkage of fertility clinic data to registry data was performed using probabilistic matching software (Automatch V4.3, MatchWare Technologies), supplemented by hand matching and checking.

The population for analysis was restricted to women having their first births (from either natural or assisted conception) because women are most likely to be in the workforce at this time. In subsequent pregnancies, women may report their usual occupation, but may not actually be in the paid workforce or may return to work in a part-time capacity.[17]

The study was approved by the ethics committees of the South Australian Department of Health, the University of Adelaide, and Flinders University. Individual patient consent was not required by the ethics committees.

*Night shift exposure*

The title of mother's usual occupation prior to and/or during pregnancy, coded using the Australian Standard Classification of Occupation version 1 (ASCO), was obtained from the State Perinatal Collection. A job exposure matrix (JEM) was applied in order to infer night shift work exposure. Job-exposure matrices provide a cross-classification of job titles and the probability of occupational exposure.[18] Details of the development of the shift work JEM are published elsewhere.[19] In a validation study, the JEM performed almost as well as job specific questionnaires in terms of reproducing an established association.[19] The shift work JEM assigned a probability of exposure to shift work involving

exposure to light at night, which was the exposure of interest for this analysis. This exposure variable was selected as it provided a stronger indicator of involvement in night and rotating shift work, which are more likely to produce circadian disruption and phase shift.[20] The JEM provided numeric probabilities of exposure for each occupational title. For the purposes of this analysis, this was recoded into a binary (exposed/unexposed) variable using the 30% cut off, an acceptable threshold according to previous studies.[19, 21] This meant that any occupation in which at least 30% of workers were exposed was considered an exposed occupation. For all analyses the comparison group was restricted to women who were in paid employment, but not exposed to night shift work. This reduced the potential for bias associated with the infertile worker effect.[22]

*Covariates*

Potential covariates were selected based on whether the existing literature indicated a demonstrated or plausible association with either night shift work or urogenital anomalies. These included maternal age (five-year age groups), maternal ethnicity (Caucasian or non-Caucasian) and socioeconomic status, which was assigned using postcode of residence and the Socio-Economic Indexes for Areas (SEIFA) produced by the Australian Bureau of Statistics.[23] Medical conditions during pregnancy (pre-existing diabetes, gestational diabetes, pre-existing hypertension, pregnancy induced hypertension, epilepsy and asthma) and fetal sex were also considered.

Preliminary analyses indicated that results for multiple births were markedly different to those for singletons, so simply adjusting for multiplicity was not an appropriate way to take this factor into account. There was insufficient power to conduct separate analyses for singletons and multiples, with models failing to converge. Therefore, product terms of the dichotomous exposures shift work and multiplicity were included in the model. That is, the risk of urogenital defects was assessed in four separate groups: multiples exposed to maternal shift work, non-shift work multiples, singletons exposed to maternal shift work, compared to a reference group of non-shift work singletons.

Maternal BMI was not recorded on the perinatal records during the study period, so it could not be examined in the main analysis, or among naturally conceived births. Maternal BMI among women conceiving a first birth with fertility treatment was assessed in sensitivity analyses, for those women whom BMI was available from fertility clinic records. Smoking was recorded on the perinatal records for only part of the study period, so sensitivity analyses were conducted using a restricted dataset containing this variable.

Treatment type was obtained from fertility clinic records. Treatment types included spontaneous conception prior to treatment initiation, minimal intervention or ovulation induction (OI) only, in vitro fertilisation (IVF), intracytoplasmic sperm injection (ISCI), intrauterine insemination (IUI), gamete intrafallopian transfer (GIFT) or use of donor oocytes. Year of birth was used as a proxy for changes in the fertility treatment protocols in place over time. Infertility diagnosis was also considered. This was defined according to one of the following categories male infertility only, endometriosis only, ovulatory infertility only, tubal infertility only, other/mixed female infertility, combined male and female infertility, idiopathic infertility.

Offspring of indeterminate or unknown sex (n=24, 0.03%) were coded as male in the analysis. This assumption was tested in sensitivity analyses by recoding these births as female and observing any changes in the results.

*Statistical analysis*

We tabulated maternal health and sociodemographic characteristics, as well as pregnancy and birth characteristics (stratified by multiplicity), by night shift exposure status, separately for each mode of conception. Chi-square tests (for categorical variables) and student's t-tests (for continuous variables) were also undertaken to provide an initial guide to the extent of confounding that might arise due to these factors. Within the fertility treatment group, we also examined the frequency of different treatment types by night shift exposure and undertook chi-squared testing to determine whether some treatment types were more commonly administered to night shift workers.

Using multivariable logistic regression, we then examined urogenital anomalies among children born to female night shift workers who conceived with fertility treatment. Although subcategories of urogenital anomalies exist, categories were combined in this analysis due to the presence of small numbers. We then examined urogenital anomalies among naturally conceived children born to female night shift workers in the general population to determine whether maternal night shift work was a risk factor in the absence of fertility treatment. For the multivariate logistic regression, maternal age and baby sex were a priori included in the adjusted models. Other potential covariates were assessed using the change in estimates approach. Covariates were included in the fully adjusted model if they produced a >10% change in the main effect estimate, or were independently associated with the urogenital anomalies, with a p-value <0.2.[24]

To further investigate the relative contribution of shift work and fertility treatment, we included an interaction term to determine whether the effect of shift work exposure was modified by treatment. The results of the interaction analysis are presented as recommended by Knol & Vanderweele,[25] with separate effects of night shift work and fertility treatment, and the combined effects of these factors compared to a single reference category with the lowest risk of urogenital anomalies, i.e. a group with no night shift exposure and no treatment. The relative risk due to interaction (RERI) was used as a measure of interaction on the additive scale and the ratio of odds ratios (OR) was calculated as a measure of interaction on the multiplicative scale, both calculated according to methods described by VanderWeele & Knol.[26] A positive additive interaction is indicated by RERI >0 and a positive multiplicative interaction is indicated by a ratio of ORs >1.[26] As birth defect outcomes are relatively rare, ORs are assumed to approximate relative risk.[27]

In multivariable analyses, Bayesian data augmentation was performed to stabilize imprecise effect estimates arising from sparse data.[28] A fully Bayesian approach was used, whereby weakly informative priors were chosen for all variables in the model. Weakly informative priors were null centred with standard error approximately equal to two. A tabular approach to data augmentation was applied,

as outlined by Greenland.[29] Generalised estimating equations (GEE) with exchangeable correlation matrix structure produced crude and adjusted ORs and 95% confidence intervals (CI). This approach was required due to clustering in the data. Specifically, births resulting from multiple gestations that cannot be treated as independent observations. Standard logistic regression without Bayesian data augmentation was applied in the interaction analyses, as this was performed for singleton first births only.

All hypothesis tests were two-sided and p values < 0.05 were considered statistically significant. All data analysis was performed using Stata V.12. (StataCorp, College Station, Texas, USA).

**Results**

There were 98,359 first births (including stillbirths and terminations for defect) to women in paid employment between 1986 and 2002. Of these, 3,466 (3.5%) were conceived with fertility treatment. Urogenital anomalies detected up to age five years occurred in 24.8 per 1,000 first births conceived using fertility treatment and in 17.2 per 1,000 naturally conceived first births.

Table 1 describes the prevalence of maternal characteristics and conditions during pregnancy by night shift work exposure status for first births conceived naturally or by fertility treatment. Exposure to night shift work among women conceiving with fertility treatment was 13.1%, which was higher than among those who conceived naturally (11.4%). Women employed in occupations involving night shift, regardless of the mode of conception, were older, more likely to be Caucasian, more likely to reside in a higher socioeconomic area and less likely to smoke. Apart from average age which was statistically significant in both groups, these differences were statistically significant in the group who conceived naturally, but not those who conceived with fertility treatment. Night shift workers who conceived with fertility treatment were significantly less likely to experience pregnancy-induced hypertension.

**Table 1:** Prevalence of maternal and pregnancy characteristics by mode of conception and exposure to night shift work for women in paid employment.

| | Fertility treatment births | | | | | Naturally conceived births | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Night shift workers (n = 454) | | All other employed women (n = 3,012) | | | Night shift workers (n=10,817) | | All other employed women (n = 84,076) | | |
| | Mean | SD | Mean | SD | p-value | Mean | SD | Mean | SD | p-value |
| **Mean age (years)** | 32.9 | 4.12 | 32.4 | 4.07 | 0.04 | 28.5 | 4.4 | 27.4 | 4.79 | <0.001 |
| **Age (years)** | n | % | n | % | p-value | n | % | n | % | p-value |
| < 30 | 123 | 27.1 | 857 | 28.5 | | 7,107 | 65.7 | 60,234 | 71.6 | |
| 30-34 | 200 | 44.1 | 1,370 | 45.5 | 0.38 | 2.864 | 26.5 | 18,524 | 22.0 | <0.001 |
| 35-39 | 107 | 23.6 | 672 | 22.3 | | 741 | 6.9 | 4,680 | 5.6 | |
| >=40 | 24 | 5.3 | 113 | 3.8 | | 105 | 1.0 | 636 | 0.8 | |
| **Ethnicity** | n | % | n | % | p-value | n | % | n | % | p-value |
| Caucasian | 443 | 97.6 | 2,916 | 96.8 | 0.38 | 10,540 | 97.4 | 80,703 | 96.0 | <0.001 |
| Non-Caucasian | 11 | 2.4 | 96 | 3.2 | | 277 | 2.6 | 3,373 | 4.0 | |
| **Socioeconomic status** | | | | | | | | | | |
| Q1 (lowest quartile) | 76 | 16.7 | 540 | 17.9 | | 1,812 | 16.8 | 18,385 | 21.9 | |
| Q2 | 86 | 18.9 | 576 | 19.1 | | 2,394 | 22.1 | 20,718 | 24.6 | |
| Q3 | 121 | 26.7 | 725 | 24.1 | 0.72 | 2,922 | 27.0 | 20,556 | 24.5 | <0.001 |
| Q4 (highest quartile) | 170 | 37.4 | 1,168 | 38.8 | | 3,646 | 33.7 | 24,208 | 28.8 | |
| Missing | 1 | 0.2 | 3 | 0.1 | | 43 | 0.4 | 209 | 0.3 | |
| **Smoking (n=43,981)[a]** | | | | | | | | | | |
| Non-smoker | 343 | 83.7 | 2,268 | 82.7 | 0.63 | 3,392 | 80.0 | 27,942 | 76.3 | <0.001 |
| Smoker | 67 | 16.3 | 475 | 17.3 | | 846 | 20.0 | 8,692 | 23.7 | |

| | n | % | n | % | p | n | % | n | % | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Unavailable | 44 | 9.7 | 313 | 10.3 | | 6,579 | 60.8 | 47,442 | 56.4 | |
| **Conditions in pregnancy** | | | | | | | | | | |
| Pre-existing hypertension | 4 | 0.9 | 49 | 1.6 | 0.227 | 139 | 1.3 | 910 | 1.1 | 0.058 |
| Pre-existing diabetes | 2 | 0.4 | 10 | 0.3 | 0.714 | 27 | 0.3 | 204 | 0.2 | 0.890 |
| Asthma | 23 | 5.1 | 115 | 3.8 | 0.205 | 531 | 4.9 | 3,853 | 4.6 | 0.128 |
| Pregnancy induced hypertension | 46 | 10.1 | 458 | 15.2 | 0.004 | 1,417 | 13.1 | 10,829 | 12.9 | 0.521 |
| Gestational diabetes | 7 | 1.5 | 81 | 2.7 | 0.147 | 98 | 0.9 | 840 | 1.0 | 0.357 |

a. Routine reporting of maternal smoking on the perinatal record form did not begin until 1998. Therefore smoking data are unavailable for pregnancies occurring before this date.

SD = standard deviation.

Among all women who conceived a first birth using fertility treatment, night shift workers were significantly less likely to have a multiple birth (Table 2). Aside from elevated (but not statistically significant) rates of any congenital anomaly and urogenital anomalies, there were few differences in perinatal outcomes for fertility treatment-conceived singleton first births to night shift workers compared to non-shift workers. Among multiple first births conceived using fertility treatment, births to night shift workers were significantly more likely to involve a stillbirth and to have urogenital anomalies.

As expected, naturally conceived births overall were more likely to be born at term, have heavier birthweight and less likely to be a multiple gestation or have a congenital anomaly compared to births conceived using fertility treatment (Table 2). There was no difference in the rate of multiple gestations by night shift work exposure for naturally conceived births. Comparison of the perinatal outcomes for naturally conceived singletons by night shift exposure showed few differences, except for a small, but statistically significant, increase in birthweight for births to night shift workers compared to those born to women in paid employment who did not work night shift. Naturally conceived multiple births among women who worked night shift were significantly more likely to be born at term ($\geq$37 weeks), compared to women in paid employment who did not work night shift. There were lower rates of any congenital anomalies and urogenital anomalies among naturally conceived multiple births to night shift workers, although this did not reach statistical significance.

**Table 2:** Perinatal outcomes by mode of conception and night shift work exposure for first births to women in paid employment (n=98,359)

| | Fertility treatment births | | Naturally conceived births | |
|---|---|---|---|---|
| | Night shift workers (n=454) | All other employed women (n=3,012) | Night shift workers (n=10,817) | All other employed women (n=84,076) |
| **Singletons (%)** | 76.9 | 70.4 | 98.0 | 97.8 |
| Male births (%) | 45.3 | 49.9 | 51.8 | 51.5 |
| Stillbirth (%) | 0.9 | 1.1 | 0.6 | 0.5 |
| Birthweight, grams (mean $\pm$ sd)[ab] | 3,355 $\pm$ 484 | 3,361 $\pm$ 476 | 3,429 $\pm$ 457** | 3,409 $\pm$ 457 |
| Gestational age[a] | | | | |
| $\geq$ 37 weeks (%) | 90.4 | 90.0 | 94.4 | 93.9 |
| 32–37 weeks (%) | 7.6 | 8.4 | 5.0 | 5.3 |
| < 32 weeks (%) | 2.0 | 1.6 | 0.6 | 0.8 |
| Any congenital anomaly (%) | 10.3 | 8.1 | 6.0 | 5.9 |
| Urogenital anomaly (%) | 3.4 | 2.1 | 1.7 | 1.7 |
| **Multiples (%)** | 23.1** | 29.6 | 2.0 | 2.2 |
| Male births (%) | 56.2 | 53.8 | 44.9 | 47.0 |
| Stillbirth (%) | 8.6** | 2.6 | 3.3 | 2.1 |
| Birthweight, grams (mean $\pm$ sd)[ab] | 2,757 $\pm$ 416 | 2,693 $\pm$ 371 | 2,657 $\pm$ 330 | 2,713 $\pm$ 363 |
| Gestational age[a] | | | | |
| $\geq$ 37 weeks (%) | 42.6 | 37.6 | 51.2* | 43.0 |
| 32–37 weeks (%) | 43.6 | 50.1 | 37.2* | 44.9 |
| < 32 weeks (%) | 13.8 | 12.3 | 11.6 | 12.1 |
| Any congenital anomaly (%) | 11.4 | 7.2 | 4.2 | 7.6 |
| Urogenital anomaly (%) | 7.6** | 2.4 | 0.9 | 2.3 |

a. Excluding terminations for defect (n=309) and stillbirths (n=597). b. Term births only. Birthweight information was missing for 214 births.

* p<0.05    ** p<0.01

The type of fertility treatment received by night shift workers compared to non-night-shift workers was assessed to determine whether differential treatment was a potential explanation for the increased risk of urogenital anomalies among births in this group. As shown in Table 3, there was no significant difference in the types of treatment received by night shift exposure status.

**Table 3:** Comparison (number of births and percentage) of the type of fertility treatment used for conception of first births by night shift work exposure status for women in paid employment.

| Fertility treatment type | Night shift workers (n=454) | | All other employed women (n=3,012) | | p-value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Spontaneous[a] | 14 | 3.1 | 72 | 2.4 | 0.38 |
| Minimal intervention or OI only[b] | 69 | 15.2 | 504 | 16.7 | 0.41 |
| IVF | 156 | 34.4 | 1,001 | 33.2 | 0.64 |
| ICSI | 113 | 24.9 | 745 | 24.7 | 0.94 |
| IUI | 49 | 10.8 | 370 | 12.3 | 0.36 |
| Donor oocyte | 7 | 1.5 | 46 | 1.5 | 0.88 |
| GIFT | 46 | 10.1 | 274 | 9.1 | 0.48 |

a. Spontaneous conceptions occurring prior to commencement of treatment
b. Includes timed intercourse, semen tests, or low-dose hormonal stimulation
OI: ovulation induction, IVF: in vitro fertilization, ICSI: intracytoplasmic sperm injection, IUI: intrauterine insemination, GIFT: gamete intrafallopian transfer.

As the occurrence of multiple gestations differed significantly by shift work exposure among fertility treatment conceptions, logistic regression analyses included interaction terms for multiplicity. Analyses of first birth conceived using fertility treatment (Table 4) showed that the risk of urogenital anomalies was significantly higher among multiple births to night shift workers, OR=2.94 (95% CI 1.26-6.85) compared to singletons without maternal exposure to night shift work. The risk of urogenital defects was elevated for singleton births to night shift workers (OR=1.80 (95% CI 0.94-3.46)), although this did not reach statistical significance. There was no difference in the risk of urogenital anomalies for multiple and singleton births conceived using fertility treatment where the mother was not exposed to night shift work. These results were adjusted for sex of the baby, maternal age, ethnicity, socioeconomic status, infertility diagnosis, fertility

treatment type, pregnancy induced hypertension, pre-existing diabetes and asthma.

Investigation of the association between night shift work and urogenital anomalies among naturally conceived first births produced markedly different results. Maternal night shift work was not associated with the risk of urogenital anomalies in singleton offspring, OR=1.01 (95% CI 0.86-1.18) (Table 4). Maternal night shift work was associated with a reduced risk of urogenital anomalies in multiple offspring, but this was not statistically significant. There was elevated risk of urogenital anomalies for multiple births compared to singleton births where the mother was not exposed to night shift work. These results were adjusted for sex of the baby, maternal age, ethnicity, socioeconomic status, pregnancy induced hypertension, pre-existing hypertension, pre-existing diabetes and asthma.

An interaction term was added to the regression model to further investigate why there appeared to be an association between maternal night shift work and urogenital anomalies in babies conceived using fertility treatment, but not those who were conceived naturally. Table 5 illustrates the combined effects of night shift and fertility treatment exposure, the stratified results and the tests for additive (RERI) and multiplicative (ratio of ORs) interactions (Table 5). Here the RERI = 0.92 (p-value = 0.003), indicates a statistically significant positive additive interaction on the additive scale. The ratio of ORs = 1.77 (p=0.094). This suggests a positive multiplicative interaction, but was not statistically significant.

**Table 4:** Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between maternal exposure to light at night during shift work and urogenital defects, in first births by mode of conception and multiplicity.

| | Fertility treatment | | | | | | Natural conception | | | | | |
| | Non-shift work | | | Night shift work | | | Non-shift work | | | Night shift work | | |
| Multiplicity | N | Unadjusted OR [95% CI] | Adjusted OR [95% CI] | N | Unadjusted OR [95% CI] | Adjusted OR [95% CI] | N | Unadjusted OR [95% CI] | Adjusted OR [95% CI] | N | Unadjusted OR [95% CI] | Adjusted OR [95% CI] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Singletons | 2127 | 1.00 [reference] | 1.00 [reference] | 349 | 1.62 [0.85–3.06] | 1.80 [0.94–3.46] | 82,234 | 1.00 [reference] | 1.00 [reference] | 10,603 | 1.01 [0.87–1.18] | 1.01 [0.86–1.18] |
| Multiples | 885 | 1.12 [0.65–1.94] | 1.09 [0.63–1.90] | 105 | 3.53 [1.51–8.25] | 2.94 [1.26–6.85] | 1842 | 1.33 [0.94–1.90] | 1.34 [0.94–1.91] | 214 | 0.54 [0.11–2.62] | 0.54 [0.11–2.70] |

a. Adjusted for sex of the baby, maternal age, ethnicity, socioeconomic status, infertility diagnosis, fertility treatment type, pregnancy induced hypertension, pre-existing diabetes, asthma.
b. Adjusted for sex of the baby, maternal age, ethnicity, socioeconomic status, pregnancy induced hypertension, pre-existing hypertension, pre-existing diabetes, asthma.

**Table 5:** Adjusted odds ratios (OR) and 95% confidence intervals (CI) for urogenital anomalies in relation to maternal exposure to light at night during shift work and mode of conception and measurements of additive and multiplicative interaction among 95,072 singleton first births to women in paid employment.

| | Maternal night shift work exposure | | | | | |
| | Non-shift work | | Night shift work | | Effect of night shift within strata of mode of conception (reference = non-shift work) | |
| | OR | 95% CI | OR | 95% CI | OR | 95% CI |
|---|---|---|---|---|---|---|
| **Natural births** | 1.00 | . | 1.01 | [0.86-1.18] | 1.01 | [0.86-1.18] |
| **Fertility treatment births** | 1.18 | [0.87-1.61] | 2.11 | [1.17-3.79] | 1.74 | [0.90-3.35] |
| **Effect of fertility treatment within strata of night shift work (reference = natural conception)** | 1.19 | [0.88-1.62] | 2.00 | [1.09-3.69] | | |

**Measure of interaction on additive scale:**

RERI (relative excess risk due to interaction) [95% CI] = $OR_{11} - OR_{10} - OR_{01} + 1 = 2.11 - 1.18 - 1.01 + 1 = 0.92$ [0.63-3.2], p value = 0.003.

**Measure of interaction on multiplicative scale:**

Ratio of ORs [95% CI] = $OR_{11}/OR_{10}OR_{01} = 2.11/(1.18*1.01) = 1.77$ [0.91-3.46], p value = 0.094.

ORs adjusted for maternal age, socioeconomic status, ethnicity, pre-existing diabetes, asthma and hypertension, pregnancy induced hypertension, and sex of the baby.

To investigate whether coding of births of indeterminate or unknown sex as male had any influence on the results, the analysis among both the fertility treatment and naturally conceived groups was repeated with these coded as female. The odds ratio results for exposure to light at night remained the same (results not shown).

In sensitivity analyses, data were restricted to the period in which smoking was routinely recorded. Among naturally conceived births, including smoking status in the model minimally strengthened the effect estimates presented in Table 4, but did not change interpretation of any results. Among fertility treatment births, the analogous sensitivity analysis failed to converge. Including BMI in the models, where available for the fertility treatment group, made little difference to the magnitude of any associations.

Finally, year of birth was added to the multivariable models as a proxy for differences in the fertility treatment protocols in place over time. Addition of this variable to the model did not produce any differences in the effects estimates presented in Table 4 for fertility treatment births.

**Discussion**

This study investigated the contribution of a patient factor (maternal night shift work) and treatment modality in the development of congenital urogenital anomalies. There was significantly higher risk of urogenital anomalies among multiple first births conceived by night shift workers using fertility treatments. For singleton births conceived by night shift workers using fertility treatments, the risk was elevated, but did not reach statistical significance. These results did not appear to be related to differences in the specific type of fertility treatment received by night shift workers compared to non-night-shift workers. Among offspring conceived naturally, probable maternal exposure to night and rotating shift work had no effect on the risk of urogenital anomalies. Investigation of the interaction between use of fertility treatment and maternal night shift work

indicated an ordering of risk, whereby greatest risk of urogenital anomalies occurred among births that were jointly exposed to maternal night shift work and fertility treatment.

No other studies have looked specifically at the risk of urogenital defects among female night shift workers. Nursing is one occupation where night and rotating shift work is common and for which there has been investigation of urogenital anomalies. A case-control study of 4,915 cases and 3,027 controls found significantly higher risk of genital defects, urinary defects and birth defects overall among children of female nurses.[30] Conversely, a cohort study of 23,222 nurses did not confirm these results, finding lower rates of genital and urinary defects among children of nurses compared to the general population.[31] A caveat of these studies, and indeed our study, is that individual effects of the various hazardous exposures experienced by nurses, such as infection, solvents and shift work cannot be separated. It is also possible that nurses, given their greater knowledge of health and the healthy system, may be more inclined to seek medical assistance for fertility problems and hence undergo treatment. This is unlikely in our study as there were roughly equal numbers of nurses in the natural conception (73%) and fertility treatment (75%) groups.

Mechanistically it is possible that altered endocrinology produced by circadian misalignment in female night shift workers may contribute to the increased risk of urogenital anomalies. However in this study, urogenital anomalies were increased only among babies conceived with fertility treatment. If either altered androgen-estrogen balance or melatonin secretion were driving the association between shift work and urogenital defects, we would expect to see an effect regardless of mode of conception. However, it is possible that there is a subgroup of women are more susceptible to the reproductive effects of circadian misalignment, both when attempting to conceive and during fetal development.

Parental subfertility itself has been associated with increased risk of urogenital anomalies in offspring.[4] It has been shown previously that endometriosis and menstrual irregularities occur more frequently among women engaged in night and rotating shift work.[14, 16] Of particular interest here is a study of endometriosis

in rotating night shift workers by Schernhammer et al.[15] This study found higher rates of endometriosis among rotating shift workers, but only among those with concurrent infertility, leading the authors to raise the idea of an interaction between the pathophysiology of infertility and the physiological disturbances produced by night and rotating shift work.[15]

Greater severity of infertility among susceptible night shift workers, or the presence of menstrual disturbances produced by circadian disruption in the absence of clinical infertility, may drive more night shift working women towards fertility treatment. This may increase exposure to invasive treatments, such as intracytoplasmic sperm injection among these women, which has been shown to increase the risk of birth defects in general, as well as urogenital and urogenital defect subtypes.[32] We did not observe any significant variation in the types of fertility treatment received by night shift exposure status. However, we were unable to consider individual steps in the treatment process, such as the stimulation protocol used for ovulation induction. The risk of urogenital defects may vary by the type of agent used for ovarian stimulation.[32]

Finally, it is also possible that there is an unmeasured factor, such as a gene, which conveys increased susceptibility to the effects of circadian disruption on infertility among women, as well as a susceptibility to urogenital defects in their offspring. The fertility treatment allows these women to have a pregnancy, when they otherwise would not be able to conceive. Thus, being able to use treatments to conceive unmasks a susceptibility to urogenital anomalies that would not otherwise be observed.

The linked datasets used in this analysis provided detailed information regarding reproductive outcomes and potential confounders, however the use of routinely collected data has some limitations. There was limited occupational information available and as we do not have individual-level shift work information for each woman, it is likely that there are variations in the types, intensity and duration of night and rotating shift work in this group. This may influence the severity of circadian disruption and infertility,[11] and may also explain why some women required fertility treatment to conceive and therefore have increased risk of

urogenital anomalies among their offspring. Use of routine data collections also meant that it was not possible to consider the prevalence of reproductive health conditions, such as menstrual irregularities, endometriosis in women who conceived naturally. We also did not know the time to pregnancy for natural conceptions. Further, although we did have information on diagnosis in the fertility treatment group, we lacked power to look at how interactions between the specific infertility diagnoses and shift work influence the risk of urogenital defects.

The use of Bayesian data augmentation makes it possible to analyse rare, but important outcomes such as birth defects. However, the used of weakly informative, null-centred priors pulls results towards the null. Therefore, the estimates provided in this study are conservative. Despite accounting for sparse data, the analysis produced wide confidence intervals for several covariates.

This study demonstrates an approach to the investigation of patient and treatment factors contributing to the risk of urogenital defects in offspring conceived using fertility treatment. Maternal shift work involving exposure to light at night was significantly associated with urogenital defects in their offspring, but only among women who conceived with fertility treatment. The interaction between maternal shift work and use of fertility treatment suggests that individual susceptibility to circadian disruption and the impact of this on severity of infertility are important factors in predicting adverse outcomes, such as urogenital anomalies.

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# 7 Discussion and recommendations

## Summary

This thesis has described a program of research investigating the impact of women's night shift work on need for fertility treatment and whether this patient factor interacts with treatment factors to influence the risk of congenital urogenital anomalies in offspring.

This work began with a critical review of the epidemiological literature relating to shift work and reproductive health outcomes. Several gaps in the literature were identified. These included whether subfertility leads to greater recourse to fertility treatment among female night shift workers and the range of diagnoses among night shift workers who undergo treatment. In addition, it was apparent that there has been little investigation of congenital anomalies among offspring of night shift workers, particularly urogenital anomalies, for which there are plausible mechanisms.

Following this, a job-exposure matrix (JEM) was developed, which allowed the assessment of night shift work exposure for a large population-based cohort produced from routine data collections. While existing shift work JEMs were available, none were created specifically for application in Australia. The JEM identified a number of key occupations in which exposure to light at night, an indicator of night and rotating shift work, was probable. These included nurses, police officers and security guards. This JEM also provided the probability of exposure to several other indicators, reflecting biologically plausible mechanisms for the potential impact of shift work on health, making it a useful tool for exposure assessment in the absence of detailed job history and exposure data, particularly in the Australian context.

The shift work JEM was then applied in a subsequent study investigating the uptake of fertility treatment among female night shift workers. In applying the

JEM, occupation is used to estimate the likelihood of shift work at night, which is a proxy for exposure to light at night. Existing literature suggested that night and rotating shift work is detrimental to female reproductive health, however no previous studies had investigated the use of fertility treatment among these workers. The results of this study indicated that, overall, women with probable exposure to night shift work were more likely to access fertility treatment compared to non-shift workers. This result was attenuated by adjustment for age, but other factors such as socioeconomic status and ethnicity had little influence. Older age of these shift working women may reflect a combination of social and biological factors that delay child bearing, such as not finding a suitable partner, or shift work affecting intimacy, as well as trying to conceive naturally without success. Child bearing may also be delayed due to the duration of education and training required to qualify for an occupation such as registered nursing. However, direct effects of shift work are indicated by the differential patterns of infertility diagnosis observed, specifically, increased rates of endometriosis and menstrual irregularity among night shift workers compared to non-shift workers. These results supported the findings of existing literature on the topic.

It has been shown previously that children conceived with fertility treatment are at greater risk of adverse perinatal outcomes, including congenital anomalies.[7] There is also evidence indicating that this is not solely attributable to treatment factors, such as ovarian stimulation and gamete manipulation, but also the patient factors that lead couples to seek treatment.[64] This evidence, and the finding from the above investigation of fertility among night shift workers informed the next study, which investigated whether maternal night shift work is a patient factor that contributes to the increased risk of congenital urogenital anomalies in offspring conceived using fertility treatment. In addition to the increased incidence of this specific category of anomalies among children conceived using fertility treatment,[7] the selection of urogenital anomalies as the perinatal outcome of interest partly reflects the plausibility of mechanistic pathways linking night shift work and circadian disruption with the development of these anomalies. This study was conducted using two reference groups, one containing medically assisted conceptions, the other natural conceptions, which allowed separation of the effects of shift work and fertility treatment. Night shift work was associated

with a significantly elevated risk of urogenital defects in babies conceived using fertility treatment, but not those conceived naturally.

To investigate the extent to which this result was attributable to treatment factors, an interaction term for night shift work and fertility treatment was added to the model concerning urogenital anomalies in the whole population. The results of this analysis indicated that while night shift work alone had little impact on the risk of urogenital anomalies, when night shift work was combined with fertility treatment the risk was greater than that associated with fertility treatment alone. The finding that the effect of night shift work on urogenital anomalies is augmented by the presence of infertility has parallels with the investigation of shift work in relation to other health effects. For example, the relative risk of cardiovascular disease among shift workers is more substantially increased when other risk factors, such as obesity are also present.[207, 208]

There did not appear to be any significant differences in the type of fertility treatment received by night shift workers compared to non-shift working women (despite differences in diagnosis as seen previously). One possible explanation is that there is a sub-population of individual night shift workers that are less tolerant of circadian disruption and therefore more susceptible to fertility problems. This increased susceptibility to the effects of circadian disruption on fertility, or the exacerbation of underlying medical problems by night and rotating shift work may contribute to adverse perinatal outcomes in these women.

A second possible explanation is that women with fertility problems related to night shift work have altered responses to treatment. For example, circadian disruption of hypothalamic function may increase the risk of anovulatory infertility in the sub-population of shift workers. It is possible that the increased sensitivity to exogenous factors controlling ovulation may extend to the hormones used for ovulation induction, such that they are at increased risk of adverse outcomes. Alternatively, these women may have diminished central control of ovulation, which requires increased doses of hyperstimulation drugs placing the fetus at risk due to increased exposure.

It is possible that individual differences in tolerance and adaptation to night shift work influence the severity of subfertility and, therefore, the likelihood of seeking fertility treatment. Shift work tolerance refers to an individual's long term acceptance of shift work and has been defined as the absence of symptoms associated with circadian disruption such as gastrointestinal disturbance, sleep disturbance, fatigue and changes in mood and behaviour.[209, 210] A review of the literature by Saksvik et al.[90] identified 60 studies (including 10 longitudinal) that investigated shift work tolerance using a wide variety of measures. Although the results were variable, younger age, male gender, eveningness chronotype, more flexible circadian type and sleeping habits and personality traits that included internal locus of control and extraversion appeared to be positive predictors of shift work tolerance in most studies. In the present work, less tolerance for shift work may manifest as increased susceptibility to the effects of circadian disruption on fertility, an increased severity of subfertility or altered response to components of the fertility treatment process.

Although the frequencies of broad fertility treatment categories did not differ between night shift workers and non-shift workers, it is possible that altered severity of infertility or response to treatment dictated variations in treatment protocols that are not captured by these broad categories. For example, differences in the type of drugs used for ovarian stimulation, and the level of response in terms of oocytes retrieved could not be assessed using the current cohort dataset. Differences in the type of drugs used for ovarian stimulation may reflect changes in treatment protocols over time. As Figure 4 shows, among births conceived after fertility treatment, a larger proportion were to mothers reporting nursing as their usual occupation. This may suggest that nurses were slightly more likely to have received treatment earlier in the time period under study. However, adjustment for year of birth (a proxy for year of treatment), did not produce any change in the effect estimates for the association between night shift work and urogenital anomalies.

Finally, it is also possible that there is an unmeasured confounding factor, such as a gene, which conveys both increased susceptibility to the effects of circadian disruption on fertility among women, and susceptibility to urogenital defects in

their offspring. It is possible that fertility treatment technologies allow these women to have a pregnancy, when they otherwise would not be able to conceive. Thus, being able to use fertility treatment to conceive unmasks a susceptibility to urogenital anomalies that would not otherwise be observed.

Taken together, the findings of this work support the theory that night shift work is not good for human health, but in complicated ways. Exposure to night shift work may modulate the effects of other genetic, psychosocial and lifestyle factors that contribute to an individual's susceptibility to the reproductive effects, and potentially other health effects, of night shift work and circadian disruption. Alternatively, the effect of night shift work on reproductive health may depend on a combination of individual vulnerability, the type of shift work, and worker's degree of choice and control over their work schedule.

**Key strengths and weaknesses**

A key strength of these studies lies in the size of the dataset. Data linkage of routine datasets provided access to many more records than would be possible in a bespoke prospective study. The use of routine data collection also reduces the risk of recall bias, which has the potential to bias results when other methods of retrospective data collection are used. Furthermore, the depth and breadth of information available from fertility clinics relating to treatment and diagnosis characteristics makes this cohort unique from both an Australian and an international perspective. Nevertheless, despite the size of the cohort, the many different combinations of infertility diagnosis and treatment protocols meant that detailed analysis was not possible. A further disadvantage of routine data collections is that not all pertinent variables are collected, or collected reliably, e.g. BMI and smoking.

Job-exposure matrices are a well-accepted, and in some cases well-validated, method to extrapolate exposure from occupational data where direct measurements cannot be made.[168, 211, 212] However, the application of a JEM provides only an occupational group level estimate of shift work exposures. The frequency and duration of night and rotating shift work is important for

determining the extent and severity of circadian disruption in individual workers. For example, there is evidence that duration of rotating shift work is associated with menstrual irregularities.[96, 213] Without this individual level information, we cannot determine whether women who conceived using fertility treatment have been performing night shift work for a longer duration, and hence have more severe subfertility. In the analyses contained in this thesis, occupation is used to estimate the likelihood of shift work at night, which is a proxy for exposure to light at night. Probable exposure to light at night was assigned for women exposed to bright or medium light in working areas and/or light in their bedroom when trying to sleep. Although this question was asked in the context of their normal working area during night shift, some misclassification due to differences in interpretation of the question and the brightness of the light cannot be ruled out. Despite these limitations, this method is the most feasible approach for estimating exposure, given the size and nature of the data in this work, and ascertaining whether further research is required.

The application of Bayesian data augmentation methods allowed the study of rare, but important outcomes such as a specific type of congenital anomalies. On the other hand, the use of a fully Bayesian approach (use of priors for all covariates regardless of their individual data sparsity) and weakly informative, null-centred priors pulls results towards the null. Therefore, the estimates provided in the study of congenital urogenital anomalies among assisted conceptions are probably conservative.

It is important to consider clustering, or interdependencies, between observation in studies of fertility treatment and birth cohorts and failure to do so is likely to result in overestimation of the precision of analyses.[214] In the present set of studies, generalised estimating equations (GEE) was used to account for clustering resulting from multiple gestations. This method allows for population-level inferences, which is beneficial for information policy, but does not provide subject-specific inferences, which would be useful for informing clinical decision making and prediction.[214]

In an attempt to separate the effects of treatment and underlying subfertility in the study of congenital urogenital anomalies, the analysis was conducted in two reference groups. Comparison of the effect of maternal night shift work on the risk of urogenital anomalies in offspring conceived naturally versus those conceived with fertility treatment showed that shift work alone was not sufficient to induce urogenital anomalies. Further, the examination of types of treatment received by shift workers compared to non-shift workers showed that the observed association between shift work and urogenital anomalies in the fertility clinic cohort was not likely to be related to a treatment effect.

It must also be recognised that fertility is a characteristic of a couple, rather than an individual and when investigating fertility and perinatal outcomes, characteristics of both the male and female should be considered. In the study of uptake of fertility treatment, women were excluded from the analysis if the diagnosis was male only infertility. In the analysis of urogenital anomalies, male only infertility was adjusted for in the regression models, however there was limited information on potentially important covariates such as paternal age, occupation, smoking and BMI. Paternal age was available from the fertility clinic data for some pregnancies conceived with medical assistance, but this is not collected on the perinatal record for births at the general population level.

Similarly, maternal smoking has only been routinely collected on the perinatal record from 1998 and maternal BMI from 2003, therefore data for these variables was not available for the complete cohort. However, sensitivity analyses using data from births for which this information as available, suggested that these factors did not have had a big impact on the results.

## Recommendations

The key recommendations arising from this thesis for future research and policy are outlined below.

*Exposure assessment in epidemiological studies of shift work*

It is clear that population-based studies are required to confirm the results of the smaller clinical studies that have identified adverse health effects of shift work. However, as exemplified in the studies that comprise this thesis, detailed exposure assessment in large, registry based cohorts is challenging. Prospective studies are one option to obtain detailed exposure and outcomes information, but this is rarely feasible due to the expense and intrusiveness of such a study. Prospective studies are also unlikely to provide sufficient power to investigate rare outcomes.[214] The cost and burden of biological sampling, e.g. urine or blood melatonin, limits its application in large cohorts. Although, one more feasible example may be cortisol. Cortisol provides a marker of biological response to chronic stressors, such as shift work. Measurement of the cortisol awakening response via saliva sampling is also considered a key measure for gaining insight into inter-individual tolerance or adaptation to shift work schedules.[215] New technologies that allow measurement of hormones, including cortisol, from hair strands may provide a more affordable option and one that is more acceptable for participants.[216]

Another option would be to assess shift work exposures using job specific module questionnaires delivered by computer-assisted telephone interviewing. For example, these questionnaires are available via the OccIDEAS system and have been used to collect job and task specific information from study participants in other studies, such as the BCEES.[171, 217] If these studies are conducted retrospectively, care must be still taken to minimise recall bias.

In the absence of prospective studies, studies that are able to draw on retrospective data that contain detailed information on the shift schedule performed, including direction and speed of rotation, frequency and duration of the shift schedule are needed. This may be possible through the use of rostering and payroll data from large organisations (such as hospitals) that employ large numbers of shift workers.[218]

### *Recording and reporting of perinatal and fertility clinic data*

The collection and reporting of data from fertility clinics has improved since the period of the data analysed in this thesis. However, public reports of statistics

relating to fertility treatment still do not provide a complete picture of all fertility treatments occurring in Australia. The Australian and New Zealand Assisted Reproduction Database (ANZARD) only records data on treatment cycles involving manipulation of both the male and female gametes, i.e. ART treatments, with the exception of donor insemination.[30] Data on intrauterine insemination using partner's own sperm and treatment involving the use of ovulation induction drugs without oocyte collection is therefore unavailable. In addition, follow up of pregnancies conceived using fertility treatment is limited and varies from clinic to clinic.[30] This prohibits analysis of the safety and long term outcomes of fertility treatment. Although this is in part due to change in care providers, i.e. from fertility specialist to obstetrician, there is a need for standardised system across clinics and jurisdictions.

Public reports of fertility treatment in Australia are also unable to capture the provision of treatment outside of fertility clinics. As described above, data from a cohort of South Australian women indicated that 41% of those who sought medical assistance for fertility problems were treated with medication for ovulation induction only,[16] compared to 26% in the UK.[31] This may suggest that a proportion of Australian women who seek medical assistance for fertility problems are treated by specialists outside of specialised fertility clinics using less invasive methods. Although no existing mechanism exists to incorporate this data into ANZARD, it may be possible to access this information through data linkage with the Pharmaceutical Benefits Scheme.

Further improvements and standardised reporting mechanisms for treatment and pregnancy data would improve the outcomes for patients and their children, by providing for complete information for patient and clinical decision and policy making. It would also help to identify treatments or technologies that are not effective or even detrimental, further improving outcomes in this population.

When designing epidemiological studies, particularly those that consider the outcomes of fertility treatment and pregnancies, the ability to identify an appropriate reference group is paramount, but challenging when using routine data collections. As seen in the urogenital defects studies contained in this thesis,

the presence of an effect can vary depending on the choice of reference group that is, those who conceived with or without medical assistance. When studying the outcomes among those who conceive with medical assistance, it is important to also consider the use of an internal reference group, as this population is inherently different from the general population of fertile couples. This is highlighted by studies that have identified patient factors that contribute to adverse outcome in this group and that remain important predictors after treatment factors are taken into account. Further, a key comparison group required for the separation of patient and treatment effects is a group of subfertile couples who conceived naturally. Identification of such a reference group remains a challenge for research involving routine data collections, as care must be taken to ensure that this group is not contaminated by access to minimally invasive treatments, such as ovulation induction drugs, that may be accessible outside of fertility clinics. Subfertile couples who conceived naturally may be identified from fertility clinic records of couples who sought clinic-based treatment for infertility, but conceived spontaneously before or between treatment cycles

Linkage of more contemporary data from fertility clinics to the Perinatal Statistics Collection and the Birth Defects Register would provide an even larger sample size for future analyses and data on potentially important covariates such as smoking and BMI. This would permit a broader range of stratified analyses, including stratification by multiplicity. A larger sized dataset would also allow investigation of specific combinations of infertility diagnosis and treatment modalities as well as the effects of different ovarian stimulation protocols, embryo culture media and other factors within treatment regimens that may vary from individual to individual. Lastly, there would be greater power for further examination of patient and treatment factors through the investigation of outcomes among subfertile couples who conceived naturally and potentially sibling studies.

### *Shift work and health policies*

There is growing evidence that shift work is damaging for health. In relation to cardiovascular and metabolic health, large systematic reviews and meta-analyses

report significant elevations in coronary heart disease and type two diabetes among shift workers.[219] In addition, shift work involving exposure to light at night has been classified as a 'probable carcinogen' by the International Agency for Research on Cancer.[136] It appears that the health effects of shift work are complicated by individual differences in tolerance of night shift work and resulting symptoms of circadian disruption.[90, 220] The work contained in this thesis advances understanding of the consequences of night shift work for the reproductive health of women.

In view of the accumulated evidence, there is a need for greater consideration of these health effects in workplace policies. For many occupations and industries, it would be impractical to stop night and rotating shift work completely, but it is time for employers and regulators to consider how the effects of shift work on health can be managed and minimised. This is reportedly lacking specifically for the nursing workforce, which is concerning.[208]

For fertility and reproductive health, this could entail the development of guidelines around who works night shift and when. For example, allowing altered shift arrangements for women who are actively planning a pregnancy or when pregnancy occurs. This is particularly relevant for women who have existing concerns about the effect of their work on their fertility. Such policies are already in place in some European countries, where women are prohibited from working night shift during pregnancy and the postpartum period.[136]

Alternatively, rather than specifically targeting women planning pregnancy , a more broadly applicable approach would be to allow all workers a degree of choice around shift work schedules. Higher levels of work time control among shift workers has been associated with fewer days of absence due to long-term sickness. Further, in an intervention study, workers who were allocated to a self-rostering system, that allowed choice of work days and duties, reported fewer symptoms of circadian disruption (e.g. digestive disturbances), decreased mental distress, and improved sleep quality.[221] This would provide an individual with more control over their own work schedule and a tailored schedule based on their tolerance to shift work and family responsibilities.[221]

## Conclusion

Night and rotating shift work affects female reproductive health and wellbeing through the physiological consequences of circadian disruption. In addition, this type of work is likely to have psychosocial consequences that impact on the timing of childbearing and family life. This has important public health implications as older age of childbearing conveys risks to both mother and child through increased rates of pregnancy complications and poor neonatal outcomes. Further risks arise with recourse to fertility treatment, which is costly to individual couples, and if treatment is publicly funded, society in general. This work has also shown that interactions between maternal shift work and use of fertility treatment can produce further adverse outcomes in the form of urogenital anomalies among susceptible individuals. By highlighting the concept of individual susceptibility to circadian disruption, this work contributes further to the debate surrounding the mismatch between human biology and the structure of modern society and industry, which may be remedied by flexible workplace policies that provide greater work time control with consideration of the personal circumstances of the individual worker.

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# 9  Appendices

## Appendix 1: Supplementary information on ART treatment types

**Other ART treatment types**

Zygote intrafallopian transfer (ZIFT) arose as a modification of the GIFT technique as it allows assessment of fertilisation before transfer into the fallopian tubes.[222] One or more zygotes are transferred into the fallopian tube. This means that the fertilised oocyte is transferred before it develops into an embryo, that is, before the nuclei of the oocyte and sperm fuse together.[222] ZIFT has been discontinued in many ART centres because the added cost and complexity of the procedure could not be justified by superior effectiveness over other ART treatments.[223]

Gestational surrogacy is a situation in which a woman carries the pregnancy with an agreement to give the offspring to the intended parents.[9] This may involve gametes from the intended parents and/or donors. Surrogacy cycles represented only 0.3% of ART treatment cycles conducted in Australia in 2014.[30]

In cases where there is a known risk of specific genetic disorders, embryos may be screened using preimplantation genetic diagnosis. Preimplantation genetic diagnosis (PGD) involves the analysis of cells from oocytes, zygotes or embryos to detect specific genetic, structural or chromosomal anomalies.[9] Embryos may also be screened for lethal chromosomal abnormalities to ensure that only embryos of high quality are transferred and to improve IVF success rates.[12] The use of PGD for sex selection is prohibited unless it is to avoid the transmission of a serious genetic condition.[29] In 2011, PGD was performed in 5.4% of cycles involving fresh or thawed embryos.[30]

**Clinical indicators of reproductive outcomes in ART couples**

One advantage of studying the ART population is that very early pregnancy events can be monitored and assessed. This section briefly considers two indicators of reproductive success among couples receiving ART treatment, embryo quality and implantation failure.

*Embryo quality*

Embryo quality is one determinant of whether ART treatment results in a healthy live birth. Winter et al.[47] showed that transferring the poorest quality embryo significantly increased the risk of early pregnancy loss in a sample of women undergoing ART treatment (OR = 3.26, 95% CI 1.46–7.26). The quality of fertilised oocytes cultured *in vitro* can be assessed morphologically, that is by their physical properties. The characteristics of a high quality embryo include cleavage (division) of the fertilised oocyte into 4–5 cells on day 2 or 7–8 cells on day 3. The cells (known as blastomeres) should all be similar in size, each with a single nucleus, and there should be few cells with no nucleus (known as cytoplasmic fragments).[224] Other factors used to distinguish high quality embryos with a greater likelihood of implantation include the structural features of the fertilised oocyte, time to entering the first mitotic cleavage and the biochemical activity of cleavage-stage embryos in culture.[225]

Advancements in the development of culture media has allowed embryos to be cultured for longer *in vitro*. An embryo develops into a blastocyst 5–6 days after fertilisation and consists of about 100 cells. Allowing the embryo to develop to blastocyst stage *in vitro* improves the success of embryo transfer and implantation, as this is the stage at which embryo implantation would normally occur in spontaneous pregnancy.[225] Culturing of embryos to the blastocyst stage ensures that only higher quality embryos that are capable of surviving to a later stage are used, and provides more time for PGD.[226] As mentioned, PGD can identify genetically sound embryos that are more likely to result in successful pregnancy after transfer.[225]  Approximately 67.5% of embryo transfer cycles undertaken in Australia and New Zealand in 2014 involved blastocyst stage

embryos.[30] The live birth rate per embryo transfer cycle was 28.7% for blastocyst transfers, compared to 16.6% for cleavage stage embryo transfers.[30]

### *Implantation failure*

Implantation of the blastocyst in spontaneous pregnancies occurs around day five or six post-conception. Implantation can be defined as the attachment and subsequent penetration of the endometrium by the blastocyst.[9] Implantation is identified clinically by a rise in hCG levels.[46]. Implantation failure is therefore, the failure of the transferred embryo to attach and penetrate the endometrium. It is estimated that 85% of embryos transferred during ART treatment do not implant.[227] The definition of recurrent implantation failure varies, as it depends on factors such as the number of embryos transferred per cycle.[46] One suggested definition is the failure of three or more ART cycles despite the transfer of good quality embryos.[223]

The causes of implantation failure include decreased endometrial receptivity, embryonic defects and multifactorial causes.[223] Decreased endometrial receptivity may be caused by uterine abnormalities such as hyperplasia, immunological conditions and thrombophilia. Embryonic defects may be the result of chromosomal abnormalities in the parents, gametes and/or embryos, as well as irregularities of the zona pellucida. Multifactorial causes include cases where several aspects of the reproductive process are affected. For example, endometriosis can adversely affect the endometrial lining and embryo quality.[228] Treatment options for defects in endometrial receptivity and multifactorial disorders include surgery, pharmacotherapy and immunotherapy.[223] Treatments to overcome genetic and embryonic factors include assisted hatching (thinning or rupturing of the zona pellucida by mechanical, chemical or laser methods), PGD and transfer of blastocyst stage embryos. Although there are many other suggested treatments for the causes of implantation failure, few have been shown to consistently improve pregnancy and/or live birth rates in practice.[223]

## Appendix 2: List of original fertility treatment codes and key to recoding.

**Original fertility treatment codes**

2  IVF

3  IVF + Aneuploidy screen

4  IVF + Embryo biopsy

5  Normal IVF + AFT embryos

6  IVF + Frozen ET

7  Natural IVF

8  GIFT

9  Bromocryptine/home*

10  ZIFT or TET

11  TET

12  IVF (All AFT Embryos)

13  Microinjection

14  ICSI

15  Definitely ICSI & Control embryos

16  ICSI/Epididymal sperm

17  ICSI + Frozen sperm

18  ICSI/ Epididymal sperm/Embryo biopsy

19  ICSI + Testicular sperm

20  ICSI/Frozen epididymal sperm

21  ICSI + Frozen testicular sperm

22  ICSI with PESA

23  ICSI with PESA (Frozen)

24  ICSI + Assisted hatching

25  ICSI with PGD

26  ICSI/PESA/PGD

27  E.T.

28  FET, ICSI embryos

29  ET (Imported embryos)

30  ET (Transferred overseas)

31  Donor oocyte (Frozen)

32  Donor oocyte (Fresh GIFT)

33  Donor oocyte (Fresh)

34  Donor oocyte (Fresh) + ICSI

35  Donor oocyte (Fresh) + ICSI/ Epididymal sperm

36  Donor oocyte (Fresh) + ICSI/PESA

37  Donor oocyte + TET

38  Donor oocyte + ZIFT

39  Donor embryo

40  Husband HCG injection

41  IUI Control cycle - No insemination

42  IUI

43  IUI (Cancelled ICSI)

44  IUI (Cancelled IVF)

45  Cancelled IUI - Too many follicles

46  DI

47  DI (Cancelled IVF)

48  Pregnant on Lucrin

49  Natural IVF + ICSI

50  SCMC

51  SCMC + Clomid

52  Spontaneous

53  Pregnant on Synarel

54  Spontaneous after stopping Danazol

| 55 | Cycle tracking/Intercourse timing | 83 | IUI Not treated* |
|----|-----------------------------------|-----|-------------------|

55   Cycle tracking/Intercourse timing

56   Tubal/ovarian surgery

57   Ovulation induction

58   Clomid + Bromocryptine at home

59   Clomid at home

60   Timed intercourse (Cancelled IVF)

61   Weight loss

62   Other (see notes)

66   ? (Incomplete DI)*

67   OI (Incomplete IVF)*

68   Incomplete OI - Too many follicles*

77   Infertile

82   IVF/GIFT Not treated*

83   IUI Not treated*

85   SCMC or Cycle tracking

86   DI Not treated*

87   OI Not treated*

89   Consultation only*

99   Unknown

127   E.T. IVF

129   ET (Imported Embryos) IVF

130   ET (Transferred overseas) IVF

131   Donor Oocyte (Frozen) IVF

139   Donor Embryo IVF

227   E.T. ICSI

229   ET (Imported Embryos) ICSI

230   ET (Transferred overseas) ICSI

231   Donor Oocyte (Frozen) ICSI

239   Donor Embryo ICSI

652   Spontaneous Post Treatment

*These treatment codes do not appear in the revised coding scheme as they do not represent treatments that could directly achieve pregnancy.

**Key to revised fertility treatment coding scheme**

| New code and label | Original code |
|--------------------|---------------|
| 1 Spontaneous | 52, 54, 652 |
| 2 Minimal medical intervention | 40, 46, 50, 55, 61, 48, 53, 56, 62 |
| 3 Ovulation induction only | 41, 45, 47, 51, 57, 58, 59 |
| 4 IVF Fresh | 2, 3, 4, 5, 7, 10, 11, 12 |
| 5 IVF Frozen | 6, 27, 29, 30, 39, 127, 129, 130, 139 |
| 6 ICSI Fresh | 13-26, 49 |
| 7 ICSI Frozen | 28, 227, 229, 230, 239 |
| 8 IUI | 42-44 |
| 9 Donor oocyte | 31-38,131,231 |
| 10 GIFT | 8 |
| 11 Infertile, no treatment | 77 |
| 99 Missing | 99 |

**Appendix 3: Practical steps involved in Bayesian data augmentation**

The following outlines the steps involved in Bayesian data augmentation using null priors in practical terms. It is based on the tabular approach described by Greenland 2007.[198] A visual representation is provided in Appendix Table 1.

A record, or row of data, is added to the dataset for the exposure variable and each covariate that will be included in the final model (Appendix Table 1a). The value in the outcome variable column represents the number of cases to be added to stabilise a variable, that is, to bring the number of cases (or non-cases) up to at least five. This is determined by performing a cross tabulation separately for each variable (exposure and covariates) with the outcome variable (Appendix Table 1b). In the example in Appendix Table 1, there are only four cases of twins with a congenital anomaly, therefore a '1" is placed in the outcome column in Appendix Table 1a. In a fully Bayesian approach, a prior is added for every variable, even if the number of cases and non-cases is already ≥5. In this situation, 0.5 is placed in the outcome column. The same applies for any continuous variables. A prior may also be added for the intercept. In this example, and in the analysis contained in project four, a very weak prior was added to the intercept.

Equal numbers of cases and non-cases must be added to ensure that the priors do not bias the effect estimate. To do this, a total column is included, which is equal to two times the value in the outcome variable for each record. A "1" is then placed in the column that corresponds to the variable to which the prior applies, with "0" for all other variables. All variables must be continuous or dichotomous, that is, multi-level categorical variables must be converted into dummy variables. The resulting set of prior records are then added into the real dataset for analysis. In the real dataset, the intercept and total variables should be set to "1".

**Appendix Table 1:** This example illustrates how prior data is created for Bayesian data augmentation of a dataset where one or more variables contains sparse data.

Appendix Table 1a: Records that are added to augment a dataset.

| Shift work (X) | Maternal age (C1) | Twin (C2) | Intercept (I) | Congenital anomaly (Y) | Total (2Y) |
|:---:|:---:|:---:|:---:|:---:|:---:|
| 1 | 0 | 0 | 0 | 0.5 | 1 |
| 0 | 1 | 0 | 0 | 0.5 | 1 |
| 0 | 0 | 1 | 0 | 1 | 2 |
| 0 | 0 | 0 | 0 | 3 | 6 |
| 0 | 0 | 0 | 1 | 0.000002 | 0.000004 |

X = Exposure variable, C1 = continuous covariate, C2 = dichotomous covariate, I = Intercept, Y = outcome variable, Total = 2Y = 2 x outcome variable.

Appendix Table 1b: Cross tabulation of covariate and outcome variables.

| | Congenital anomaly | |
|:---:|:---:|:---:|
| **Twin** | **0 (no)** | **1 (yes)** |
| **0 (no)** | 50 | 15 |
| **1 (yes)** | 8 | 4 |