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Night shift work undertaken by women and fertility treatment interact to increase prevalence of urogenital anomalies in children

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KEY MESSAGES

1. What is already known about this subject?

- Night shift work is known to affect circadian rhythms, producing a cascade of perturbations in the endocrine system.
- It is plausible that night shift work undertaken by women could contribute to urogenital anomalies in offspring.
- Investigation is complicated because of evidence that night shift work impairs female fertility, and assisted conception is associated with elevated prevalence of urogenital anomalies.

2. What are the new findings?

- Maternal shift work involving exposure to light at night was significantly associated with urogenital anomalies in their offspring, but only among women who conceived with fertility treatment.
- This was not explained by differences in the types of fertility treatment administered to women who worked night shift.

3. How might this impact on policy or clinical practice in the foreseeable future?

- Individual susceptibility to circadian disruption and the impact of this on severity of infertility and perinatal outcomes requires further investigation.
- Individuals with poor tolerance of shift work may benefit from choice of shift schedule and promotion of strategies to mitigate circadian disruption.

ABSTRACT

Objective

To investigate the role of maternal night shift work in urogenital anomalies in offspring, taking into account mode of conception.

Methods

A population-based cohort was produced via linkage of three datasets pertaining to fertility treatment, pregnancy and congenital anomalies. Potential exposure of primiparous women to night shift was imputed by applying a job-exposure matrix to usual occupation. Associations between exposures and offspring urogenital anomalies were examined using logistic regression. An interaction term for maternal night shift work and mode of conception (natural or assisted) was included, while adjusting for covariates including maternal age, fetal sex and multiplicity.

Results

A statistically significant multiplicative interaction (β=0.74, p=0.01) was observed between maternal night shift work and fertility treatment in relation to urogenital anomalies among first births. For natural conceptions, maternal night shift work was not associated with offspring urogenital anomalies (OR=0.99, 95% CI 0.84-1.15). Where a birth arose from fertility treatment, urogenital anomalies were significantly higher among births to night shift workers compared to day workers (OR=2.06, 95% CI 1.20-3.54). This was not due to differences in the type of fertility treatment received.

Conclusions

Women who worked night shift did not have offspring with increased prevalence of urogenital anomalies if they conceived naturally. When night shift workers conceived with fertility treatment, the prevalence of urogenital anomalies in offspring was elevated. It is

62 possible that these women had the greatest exposure to night shift work, or least tolerance for
63 this work schedule, or heightened sensitivity to hormonal aspects of fertility treatment.

INTRODUCTION

Night and rotating shift work usually involve exposure to light at night, known to affect circadian rhythms, producing a cascade of perturbations in the endocrine system. This could have consequences for the reproductive health of women and for the development of a fetus in the womb.

To elaborate, the suprachiasmatic nucleus in the hypothalamus relays circadian information to other central and peripheral circadian oscillators via regulation of clock-gene expression and neuroendocrine signalling,[1, 2] such as the rhythmic secretion of melatonin by the pineal gland.[3] Circadian clock-gene expression occurs in several reproductive tissues including the ovary, which may explain why alterations in endogenous levels of other hormones, including estrogen, have been observed among shift workers.[4, 5] Furthermore, there is some evidence that shift work adversely affects female fertility,[6, 7] which may lead to greater use of fertility treatment by these women.[8]

During pregnancy, melatonin is important as an antioxidant and in regulating the fetal circadian rhythm.[9] Beyond disrupting this rhythm, night shift work has the potential to interfere with aspects of fetal development that occur in a hormone-dependent manner, notably the male urogenital system.[10] Urogenital anomalies occur among both males and females, affecting up to 16 per 1000 births.[11] However, a higher prevalence of both urinary and genital anomalies among males has been reported in several studies.[12-14]

Previously, a systematic review considered urogenital anomalies among offspring of women employed in health care, an industry where night shift work is common.[15] Of the four included studies, three studies found no association,[16-18] and one study found a protective

association.[19] However, an earlier case-control study found significantly higher relative risk of genital system (RR = 1.61, 95% CI 1.03-2.53) and urinary system anomalies (RR = 3.43; 95% CI 1.41-8.34) among children born to nurses.[20]

To date, only one of the published studies has considered whether conception was natural or assisted by fertility treatment.[18] This is an important consideration, since fertility treatment has been identified as a risk factor for genital and urinary tract anomalies.[10, 21] Therefore, our aim was to investigate the role of maternal night shift work in urogenital anomalies in offspring, using a population-based data linkage study, taking into account mode of conception.

MATERIALS AND METHODS

Data sources and study population

As described previously,[22] the study cohort was assembled by linking data on all patients undergoing fertility assessment and treatment in South Australia (SA) with two registries, the SA Perinatal Registry and SA Birth Defects Registry. The study population thus comprised all live births, fetal deaths, and terminations (after 20 weeks) occurring among women residing in SA between 1986 and 2002 (n=327, 369).

Night shift work

The title of the mother's usual occupation prior to and/or during pregnancy was recorded in the Perinatal Registry, coded using the Australian Standard Classification of Occupation (version 1). A job exposure matrix was applied in order to infer night shift work exposure. Details of the development and validation of this job exposure matrix have been published

elsewhere.[23] Using the matrix, a probability of exposure to light at night can be assigned to each occupation, which provided an indicator of involvement in night shift work or rotating shift work involving nights. Occupations in which at least 30% of workers reported exposure to light at night, an optimal threshold as determined in previous studies,[24] were labelled "night shift workers". Those without this were assumed to be day workers.

Congenital anomalies

Structural, biochemical, chromosomal and other congenital anomalies are reported to the SA Birth Defects Register and classified according to the British Paediatric Association Modification of the International Classification of Diseases 9th Revision (ICD-9 BPA). Anomalies are reportable until a child's fifth birthday, thus are not limited to those readily detected in the neonatal period. Minor anomalies are excluded from the register unless they are disfiguring or require treatment (thus, for example, hydrocoele testis is not included). All codes relating to urogenital anomalies, ICD-9 BPA 75200 to ICD-9 BPA 75399 were considered as outcomes in the present study.

Other variables

Mode of conception is a known risk factor for urogenital anomalies.[10] Where conception occurred with clinical assistance, it was further classified as minimal intervention (encompassing timed intercourse, semen tests, or low-dose hormonal stimulation), ovulation induction (OI) only, in vitro fertilisation (IVF), intracytoplasmic sperm injection (ISCI), intrauterine insemination (IUI), gamete intrafallopian transfer (GIFT) or use of donor oocytes. We excluded 400 births for which the mode of conception was unclear. These births had infertility noted on the birth record but no corresponding fertility clinic record.

Potential covariates were identified for inclusion if existing literature supported a demonstrated or plausible association with either night shift work or offspring urogenital anomalies. Covariates obtained from the Perinatal Registry were maternal age (classified into five-year age bands), maternal ethnicity (Caucasian or non-Caucasian) and socioeconomic status, which was assigned based on postcode of residence and the Socio-Economic Indexes for Areas.[25] Medical conditions before and during pregnancy (pre-existing diabetes, gestational diabetes, pre-existing hypertension, and pregnancy induced hypertension), multiple pregnancy and fetal sex were also considered. Routine recording of maternal smoking on the perinatal record began in 1998. Therefore, smoking data was available for only 45% of births in the study period. Maternal body mass index (BMI) was not recorded in the perinatal records during the study period but was available for around three quarters of fertility treatment patients.

Statistical analysis

The population for these analyses was restricted to primiparous women in paid employment (Figure 1). This selection criteria increases the likelihood of participants being employed in their designated usual occupation around the time of conception, reducing the potential for bias associated with the 'infertile worker' effect.[26] In brief, the great majority of Australian women return to work part-time after they have a child,[27] so work-related exposures are quite different from those of childless or primiparous women.

We tabulated maternal health and sociodemographic characteristics, as well as pregnancy and birth characteristics stratified by night shift work exposure, mode of conception and multiplicity. Chi-square tests (for categorical variables) and Student's t-tests (for continuous

variables) were undertaken to provide an initial guide to associations as well as the extent of confounding that might occur.

We compared urogenital anomalies among children born to women who worked night shift with the corresponding prevalence for women who were day workers, using multivariable logistic regression. An interaction term was included to account for the multiplicative joint effects of night shift work and mode of conception. Although subcategories of urogenital anomalies exist, categories were combined in this analysis due to the presence of small numbers for some cells.

For the multivariate logistic regression, maternal age, fetal sex and multiplicity were *a priori* included to produce adjusted models. Other potential covariates were assessed for inclusion and retained in a fully adjusted model if they were independently associated with the urogenital anomalies with a p-value of less than 0.2 or produced a change of at least 10% in the main effect estimate.[28] Covariates were not included in the models if there were no exposed cases among births with urogenital anomalies.

Generalised estimating equations (GEE) with exchangeable correlation matrix structure were applied to account for clustering in the data, specifically consecutive pregnancies to the same mother and births resulting from multiple gestations that cannot be treated as independent observations. 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; as no differences were observed, these results are not presented. Sensitivity analyses were performed using a restricted dataset

containing maternal smoking data. Sensitivity analyses were also performed excluding women who were nurses.

Within the group that received fertility treatment, we examined the frequency of specific treatment types according to night shift exposure and used a chi-square test to determine whether any treatment types were more commonly administered to night shift workers than day workers. We also compared BMI for night shift and day workers who conceived with fertility treatment.

In addition to examining the distribution of treatment types by night shift exposure, pregnancy outcomes including fetal death, overall congenital anomalies and urogenital anomalies were tabulated by night shift exposure, treatment type and multiplicity. This is presented descriptively for clinical interest, as data were too sparse for statistical analysis.

All hypothesis tests were two-sided and p values < 0.05 were considered statistically significant. All data analysis was performed using Stata V.14. (StataCorp, College Station, Texas, USA).

Ethical approval

The study was approved by the South Australian Department of Health Human Research Ethics Committee (ref. no. 19 012 006), the University of Adelaide Human Research Ethics Committee (ref. no. H-002-2005), and Flinders Clinical Research Ethics Committee (ref. no. 78/02). Individual-level consent Individual patient consent was not required by the ethics committees.

RESULTS

In total, there were 98,759 primiparous women in paid employment who gave birth during the study period. Occupations for 11,271 (11.5%) women were likely to have involved night shift work. Over half of the night shift workers were nurses, with other occupations including police officers and air transport support workers (as described elsewhere).[8]

Births conceived with fertility treatment comprised 3,466 (3.5%). There were relatively more women who worked night shift in the group that conceived with fertility treatment (13.1%) than among women in paid employment who conceived naturally (11.4%).

Table 1 describes maternal and pregnancy characteristics for women grouped according to their night shift work exposure status and whether or not they conceived naturally. Women employed in occupations involving night shift tended to be older, and were more likely to be Caucasian and to reside in a higher socioeconomic area and somewhat less likely to smoke. These differences were most pronounced, and statistically significant, within the group that conceived naturally.

229 **Table 1: Maternal and pregnancy characteristics for primiparous women in paid employment, stratified exposure to night shift work**
230 **and mode of conception.**

	Naturally conceived births					Births from fertility treatment				
	Night shift workers (n = 10,817)		Day workers (n = 84,076)			Night shift workers (n = 454)		Day workers (n = 3,012)		
	n	%	n	%	p-value	n	%	n	%	p-value
Age (years)										
< 30	7,107	65.7	60,234	71.6	<0.001	123	27.1	857	28.5	0.38
30-34	2,864	26.5	18,524	22.0		200	44.1	1,370	45.5	
35-39	741	6.9	4,680	5.6		107	23.6	672	22.3	
≥40	105	1.0	636	0.8		24	5.3	113	3.8	
Ethnicity										
Caucasian	10,540	97.4	80,703	96.0	<0.001	443	97.6	2,916	96.8	0.38
Non-Caucasian	277	2.6	3,373	4.0		11	2.4	96	3.2	
Socioeconomic status										
Q1 (lowest quartile)	1,812	16.8	18,385	21.9	<0.001	76	16.7	540	17.9	0.72
Q2	2,394	22.1	20,718	24.6		86	18.9	576	19.1	
Q3	2,922	27.0	20,556	24.5		121	26.7	725	24.1	
Q4 (highest quartile)	3,646	33.7	24,208	28.8		170	37.4	1,168	38.8	
Missing	43	0.4	209	0.3		1	0.2	3	0.1	
Smoking (n = 44,025)[a]										
Non-smoker	3,392	80.0	27,942	76.3	<0.001	343	83.7	2,268	82.7	0.63

Smoker	846	20.0	8,692	23.7		67	16.3	475	17.3	
Unavailable	6,579	60.8	47,442	56.4		44	9.7	269	8.9	
Pre-pregnancy medical conditions										
Hypertension	139	1.3	910	1.1	0.06	4	0.9	49	1.6	0.23
Diabetes	27	0.3	204	0.2	0.89	2	0.4	10	0.3	0.71
Asthma	531	4.9	3,853	4.6	0.13	23	5.1	115	3.8	0.21
Conditions in pregnancy										
Pregnancy induced hypertension	1,417	13.1	10,829	12.9	0.52	46	10.1	458	15.2	<0.01
Gestational diabetes	98	0.9	840	1.0	0.36	7	1.5	81	2.7	0.15

a. Routine reporting of maternal smoking on the perinatal record form commenced in 1998.

Table 2 describes perinatal outcomes stratified by maternal shift work status and mode of conception. There was considerable variation in the proportion of births that were male, ranging from 44.9 per cent (multiples conceived naturally to night shift workers) to 57.1 per cent (multiples conceived with fertility treatment to night shift workers). Caution is thus required in interpreting the unadjusted comparisons presented in Table 2.

237 **Table 2: Perinatal outcomes for births to primiparous women in paid employment, stratified by night shift work exposure and mode of**
238 **conception.**
239

	Naturally conceived births		Births from fertility treatment	
	Night shift workers (n = 10,817)	**Day workers (n = 84,076)**	**Night shift workers (n = 454)**	**Day workers (n = 3,012)**
Singletons n (%)	10,603 (98.0)	82,234 (97.8)	349 (76.9)	2,127 (70.6)
Male births n (%)	5,491 (51.8)	42,349 (51.5)	158 (45.3)	1,063 (49.9)
Fetal deaths per 1000 births	5.8	5.2	8.6	11.3
Birth weight in grams (mean ± sd)[ab]	3,429 ± 457**	3,409 ± 457	3,355 ± 484	3,361 ± 476
Gestational age[a] n (%)				
≥ 37 weeks	9,924 (94.4)	76,581 (93.9)	311 (90.4)	1,881 (90.0)
32–36 weeks	527 (5.0)	4,287 (5.3)	26 (7.6)	175 (8.4)
< 32 weeks	64 (0.6)	673 (0.8)	7 (2.0)	34 (1.6)
Any congenital anomalies per 1000	60.3	58.6	103.1	81.3
Urogenital anomalies per 1000	17.3	17.0	34.4	21.2
Multiples[a] n (%)	214 (2.0)	1,842 (2.2)	105 (23.1)**	885 (29.4)
Male births n (%)	96 (44.9)	869 (47.2)	60 (57.1)	478 (54.0)
Fetal deaths per 1000 births	32.7	21.2	85.7**	26.0
Birth weight, grams (mean ± sd)[bc]	2,657 ± 330	2,713 ± 363	2,757 ± 416	2,693 ± 371
Gestational age[a] n (%)				
≥ 37 weeks	106 (51.2)*	775 (43.0)	40 (42.6)	324 (37.6)

32–36 weeks	77 (37.2)*	809 (44.9)	41 (43.6)	431 (50.1)
< 32 weeks	24 (11.6)	217 (12.1)	13 (13.8)	106 (12.3)
Any congenital anomalies per 1000	42.1	76.0	114.3	72.3
Urogenital anomalies per 1000	9.3	22.8	76.2**	23.7

sd = standard deviation. * p<0.05 ** p<0.01

a 98% of naturally-conceived multiple births and 91% of multiple births from fertility treatment were twins.

b Excluding terminations for defect (n=309) and fetal deaths (n=597)

c Term births only. Birthweight information was missing for 214 births.

Among births conceived naturally (Table 2), perinatal profiles for singletons were similar when bivariate comparisons were made between those exposed and not exposed to shift work *in utero*, apart from a small difference in birth weight. Around two percent of natural conceptions were multiple gestations (98% of these comprising twins, 2% higher order multiples), and this prevalence did not appear to vary with shift work exposure. Where the mother worked night shift, multiple pregnancy was significantly less likely to involve preterm birth. Variation in the prevalence of congenital anomalies and urogenital anomalies among multiple births conceived naturally was not statistically significantly.

Among births arising from fertility treatment (Table 2), perinatal profiles for singletons were unrelated to shift work exposure. There was considerable variation in the prevalence of congenital anomalies and urogenital anomalies but differences were not statistically significant in bivariate analysis. Around a quarter of births arising from fertility treatment were multiple gestations (91% twins, 9% higher order multiples), with multiples more likely to occur where the mother undertook day work. Where a mother worked night shift and had a multiple birth arising from fertility treatment, compared to mothers who worked days and had a multiple birth arising from fertility treatment, prevalence of fetal death and of urogenital anomalies were elevated.

Maternal age, fetal sex and multiplicity were included in a multivariate logistic regression analysis; other covariates that met the criteria for inclusion in the model were ethnicity, socioeconomic status, pre-pregnancy diabetes, gestational diabetes, pre-pregnancy hypertension and pregnancy induced hypertension (Table 3). A multiplicative interaction between night shift work and mode of conception in relation to the outcome of urogenital anomalies in offspring was statistically significant (β=0.74, SE=0.29, p=0.01). Among

naturally conceived first births, maternal night shift work was not associated with urogenital anomalies in offspring when other factors were considered (OR = 0.99, 95% CI 0.84 - 1.15). Where conception arose from fertility treatment, the odds of urogenital defects was elevated among births to night shift workers compared to day workers (OR = 2.06, 95% CI 1.20 - 3.54).

Table 3: Examination of associations between maternal night shift work and urogenital anomalies in offspring by mode conception.

	Day work	Night shift work	Night shift work vs Day work[a]	
Mode of conception	**n**	**n**	**Unadjusted OR [95% CI]**	**Adjusted [b] OR [95% CI]**
Natural	84,076	10,817	1.00 [0.86–1.17]	0.99 [0.84–1.15]
Fertility treatment	3,012	454	1.95 [1.14–3.33]	2.06 [1.20–3.54]

a. Odds ratios derived from logistic regression models that included an interaction term for shift work and mode conception. Unadjusted interaction coefficient β=0.67, SE=0.29, p=0.02. Adjusted interaction coefficient β=0.74, SE=0.29, p=0.01.

b. Adjusted for maternal age, fetal sex, multiplicity, ethnicity, socioeconomic status, pre-pregnancy diabetes, gestational diabetes, pre-pregnancy hypertension and pregnancy induced hypertension.

The type of fertility treatment received by night shift and day workers was compared to see whether this was a potential explanation for differences in the prevalence of urogenital anomalies in offspring (Table 4). Around a third of women conceived with IVF and a quarter with ICSI. There was no statistically significant difference in the types of treatment received by night shift exposure status. Among women who conceived with fertility treatment, maternal BMI was 25.1 kg/m^2 for night shift workers and 24.6 kg/m^2 for day workers (p=0.071).

Table 4: Type of fertility treatment used by primiparous women to achieve conception, stratified by night shift exposure.

	Night shift workers (n=454)		Day workers (n=3,012)	
Fertility treatment type	**n**	**%**	**n**	**%**
Minimal intervention	57	12.6	367	12.2
Ovulation induction only	26	5.7	209	6.9
Intrauterine insemination	49	10.8	370	12.3
Gamete intrafallopian transfer	46	10.1	274	9.1
In vitro fertilisation	156	34.4	1,001	33.2
Intracytoplasmic sperm injection	113	24.9	745	24.7
Donor oocyte	7	1.5	46	1.5

Chi-square = 2.24, df = 6, p = 0.90.

We attempted to investigate whether specific treatment modalities and multiplicity were associated with an excess of urogenital defects for night shift workers but data were sparse. Available data are provided in a supplementary table (Supplementary Table 1).

Sensitivity analyses were undertaken. When data were restricted to birth records in which maternal smoking was available (with a loss of 55% of births), smoking was not associated with urogenital anomalies (OR=0.89, 95% CI 0.74-1.06), and the main results were largely unaltered: the multiplicative interaction term was (β=0.716, SE=0.31, p=0.02) with an odds ratio for the natural conception group of 1.09 (95% CI 0.86-1.38) and 2.57 (95% CI 1.56-4.25) for the fertility treatment group. When data from nurses was removed (with a loss of 73% of births to shift workers) the multiplicative interaction term was somewhat diminished and no longer statistically significant (β=0.663, SE=0.56, p=0.23). The odds ratios were 1.03 (95% CI 0.78 – 1.37) for the natural conception group and 2.28 (95% CI 0.82-6.37) for the fertility treatment group.

DISCUSSION

This study demonstrated an interaction between maternal night shift work and the use of fertility treatment to conceive in relation to the prevalence of urogenital anomalies in first births. This was not explained by differences in the types of fertility treatment administered to women who worked night shift.

Interest in the occurrence of urogenital anomalies among births to nurses has been motivated by concern about the range of potential chemical, biological and physical exposures in the health care setting overall. A case-control study in the United States comprising 4,915 cases and 3,027 controls reported significantly higher prevalence of any congenital anomalies, genital anomalies and urinary anomalies among children of female nurses.[20] This study did not consider mode of conception.

A recent systematic review by Warembourg et al. identified four relevant studies (three cohort, one case-control) published between 2000-2015.[15] Three of the included studies found no association between maternal healthcare work and offspring urogenital anomalies.[16-18] The fourth study, a cohort study of 23,222 nurses, found that the prevalence of genital and urinary anomalies among children of nurses was significantly lower than that in the general population.[19] However, that study compared the rate of anomalies in nurses to rates in the general population standardised for year of birth, rather than to a defined comparison group, thus, it was not possible to adjust for potential confounding factors. Only one of the four included studies took into account mode of conception, finding that it had no

impact on the results. This study included first births to 5976 health care workers and 60,890 other workers form the Danish National Birth Cohort, with a prevalence of infertility treatment of 8.1% and 7.1% respectively.[18] Within the systematic review no study had specific information on work schedules.[15]

While we also do not have information on actual work schedules, an advantage of our study is that we included potential night shift workers from other industries who are unlikely to be exposed to the same infections, solvents and other hazards experienced by nurses. We undertook sensitivity analysis in which nurses were excluded. Changes in effect sizes in the model were modest, although statistical significance was not maintained, suggesting a loss of statistical power rather than altered relationships. This provides some indication of similar patterns across industries, but this needs investigation in a larger sample.

Our results concur with existing research indicating that urogenital anomalies are more common among males than female births.[12-14] Detection bias may contribute to this finding since the male sex organs are located externally, so anomalies may be more readily identified during routine examinations of males relative to females. However, congenital anomalies of the kidney and urinary tract, which may not be so easily detected, have also been shown to occur more frequently in males.[29] Our findings also confirm that urogenital anomalies are more common among babies conceived with fertility treatment.[22, 30, 31] While detection bias might contribute to this through greater treatment seeking of mothers on behalf of these children, or greater health care needs, the fact that anomalies were reported up to the child's fifth birthday mitigates against this. The variability in the ratio of males to females born after assisted reproductive treatments is consistent with published work showing this is affected by

specific components of treatment such as embryo selection parameters, culture media, and timing of embryo transfer.[32-34]

Mechanistically it is possible that altered endocrinology produced by circadian misalignment in female night shift workers may contribute to the increased prevalence of urogenital anomalies in offspring.[9, 10] However, if either altered androgen-estrogen balance or melatonin secretion were driving the association between shift work and urogenital anomalies, we might expect to see an effect regardless of mode of conception. Instead, urogenital anomalies were increased only when conception involved infertility treatment.

This might be explained by differences between night shift workers who required fertility treatment to conceive and their co-workers. An important possibility is differences in shift schedules entailing greater duration and/or intensity of shift work, hence greater interference with reproductive function, among those who required fertility treatment. A study of endometriosis in rotating night shift workers by Schernhammer et al. found higher rates of endometriosis among rotating shift workers, but only among those with concurrent infertility.[35] This led 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. Although we found an interaction, we cannot address this specific proposition as we do not have individual-level information on shift schedules. Another possibility relates to tolerance of night shift work, which has been shown to vary between individuals. Those with poor tolerance have greater sleep disturbance, fatigue, low mood, irritability and other symptoms, and could also experience greater endocrine disturbance.[36, 37] Again, we lack specific information relevant to this.

Elsewhere we showed that women undertaking night shift work who receive fertility treatment are more likely than other women receiving treatment to have menstrual irregularity.[8] It is possible that some of these women have menstrual disturbances produced by circadian disruption, in the absence of underlying clinical infertility. There was no difference between night shift workers and other women in the type of treatment received, with over half of conceptions occurring through IVF or ICSI. These are invasive treatments that involve manipulation of gametes and are associated with increased prevalence of any congenital anomalies as well as urogenital anomalies.[31] Over time, there has been variation in steps within the treatment process, such as the stimulation protocol, although it is hard to see how such variation could be systematically related to a woman's occupation.

Strengths of this study include the use of large, population-based datasets, which provided over 98,000 first births for analysis. The SA Birth Defects Register provides high quality information on congenital anomalies diagnosed up to age five years, allowing ascertainment beyond those detectable at birth. Linkage of fertility clinic data provided information on specific treatment modalities for conception, reducing the potential for confounding by treatment type. The size of the dataset also meant we were able exclude women who were nurses in sensitivity analyses, reducing the likelihood that our results were influenced by the multiple hazardous exposures of nurses.

This study also has several limitations. As mentioned, we did not have individual-level information on shift schedules for women, so we were unable to investigate possible roles of intensity and duration of night shift work. Use of occupational title to impute night shift work involves a degree of misclassification of exposure. As such misclassification occurs independently of outcome status, i.e. non-differentially, in this instance our effect estimates

are likely to be conservative. We only had data on smoking for a subset of women. However, results of our sensitivity analysis aligned with previous studies that suggest no association between maternal smoking and urogenital anomalies.[10, 21] High maternal BMI has been identified as a risk factor for urogenital anomalies,[10, 21] and is associated with shift work. While average BMI did not vary significantly by shift work status in the fertility treatment group, we did not have information on BMI where women conceived naturally, so could not fully consider potential confounding from BMI in the analyses.

Maternal shift work involving exposure to light at night was significantly associated with urogenital anomalies 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 may be important factors in determining adverse outcomes, such as urogenital anomalies.

AUTHOR CONTRIBUTIONS

RCF, VMM and MJD designed the study; RCF carried out statistical analyses; KJW provided statistical expertise; RCF and VMM drafted the manuscript; all authors interpreted the study results and contributed with manuscript revisions and approved the final version of the manuscript.

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440

441 **COMPETING INTERESTS**

442 None declared.

443

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451

452 **DATA AVAILABILITY STATEMENT**

453 The authors do not have permission to share the data as they were provided specifically for
454 the scope of research as approved by the ethics committees. Requests to access these datasets
455 should be directed to https://www.santdatalink.org.au/.

456

457 **REFERENCES**
458

1 Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annu Rev Physiol* 2010;**72**:517-549.

2 Gamble KL, Resuehr D, Johnson C. Shift work and circadian dysregulation of reproduction. *Front Endocrinol (Lausanne)* 2013;**4**:10.3389/fendo.2013.00092.

3 Haus E, Smolensky M. Biological clocks and shift work: Circadian dysregulation and potential long-term effects. *Cancer Causes and Control* 2006;**17**:489-500.

4 Gómez-Acebo I, Dierssen-Sotos T, Papantoniou K et al. Association between exposure to rotating night shift versus day shift using levels of 6-sulfatoxymelatonin and cortisol and other sex hormones in women. *Chronobiology International* 2015;**32**:128-135.

5 Schernhammer ES, Rosner B, Willett WC et al. Epidemiology of Urinary Melatonin in Women and Its Relation to Other Hormones and Night Work. *Cancer Epidemiol. Biomarkers Prev.* 2004;**13**:936-943.

6 Fernandez RC, Marino JL, Varcoe TJ et al. Fixed or rotating night shift work undertaken by women: implications for fertility and miscarriage. *Semin Reprod Med* 2016;**34**:74-82.

7 Stocker LJ, Macklon NS, Cheong YC et al. Influence of shift work on early reproductive outcomes: a systematic review and meta-analysis. *Obstet Gynecol* 2014;**124**:99-110.

8 Fernandez RC, Moore VM, Marino JL et al. Night shift among women: is it associated with difficulty conceiving a first birth? *Frontiers in Public Health* 2020;**8**:676.

9 Reiter RJ, Tan DX, Korkmaz A et al. Melatonin and stable circadian rhythms optimize maternal, placental and fetal physiology. *Human Reproduction Update* 2014;**20**:293-307.

10 van der Zanden LFM, van Rooij IALM, Feitz WFJ et al. Aetiology of hypospadias: a systematic review of genes and environment. *Human Reproduction Update* 2012;**18**:260-283.

11 Gibson CS, Scott H, Rice R et al. Birth Defects in South Australia 2012, Adelaide: SA Birth Defects Register, Women's and Children's Health Network 2015.

12 Lary JM, Paulozzi LJ. Sex differences in the prevalence of human birth defects: A population-based study. *Teratology* 2001;**64**:237-251.

13 Sokal R, Tata LJ, Fleming KM. Sex prevalence of major congenital anomalies in the United Kingdom: A national population-based study and international comparison meta-analysis. *Birth Defects Research Part A: Clinical and Molecular Teratology* 2014;**100**:79-91.

14 Tennant PWG, Samarasekera SD, Pless-Mulloli T et al. Sex differences in the prevalence of congenital anomalies: A population-based study. *Birth Defects Research Part A: Clinical and Molecular Teratology* 2011;**91**:894-901.

15 Warembourg C, Cordier S, Garlantézec R. An update systematic review of fetal death, congenital anomalies, and fertility disorders among health care workers. *American Journal of Industrial Medicine* 2017;**60**:578-590.

16 Dimich-Ward H, Le Nhu D, Beking K et al. Congenital anomalies in the offspring of nurses: association with area of employment during pregnancy. *International Journal of Occupational and Environmental Health* 2011;**17**:195-201.

17 Wiener-Megnazi Z, Auslender R, Dirnfeld M. Advanced paternal age and reproductive outcome. *Asian Journal of Andrology* 2012;**14**:69-76.

18 Morales-Suarez-Varela M, Kaerlev L, Zhu JL et al. Hospital work and pregnancy outcomes: a study in the Danish National Birth Cohort. *Int J Occup Environ Health* 2009;**15**:402-9.

506 19 Arbour LT, Beking K, Le ND et al. Rates of congenital anomalies and other adverse
507 birth outcomes in an offspring cohort of registered nurses from British Columbia, Canada.
508 *Canadian Journal of Public Health* 2010;**101**:230-234.
509 20 Matte TD, Mulinare J, Erickson JD. Case-control study of congenital defects and
510 parental employment in health care. *American Journal of Industrial Medicine* 1993;**24**:11-23.
511 21 Groen in 't Woud S, Renkema KY, Schreuder MF et al. Maternal risk factors involved
512 in specific congenital anomalies of the kidney and urinary tract: A case–control study. *Birth*
513 *Defects Research Part A: Clinical and Molecular Teratology* 2016;**106**:596-603.
514 22 Davies MJ, Moore VM, Willson KJ et al. Reproductive technologies and the risk of
515 birth defects. *N Engl J Med* 2012;**366**:1803-1813.
516 23 Fernandez RC, Peters S, Carey RN et al. Assessment of exposure to shiftwork
517 mechanisms in the general population: the development of a new job-exposure matrix. *Occup*
518 *Environ Med* 2014;**71**:723-729.
519 24 Siemiatycki J, Dewar R, Richardson L. Costs and statistical power associated with
520 five methods of collecting occupation exposure information for population-based case-
521 control studies. *American Journal of Epidemiology* 1989;**130**:1236-1246.
522 25 Census of population and housing: Socio-Economic Indices for Areas (SEIFA),
523 Canberra: Australian Bureau of Statistics 2006.
524 26 Joffe M. Biases in research on reproduction and women's work. *Int J Epidemiol*
525 1985;**14**:118-123.
526 27 Australian Bureau of Statistics. Pregnancy and work transitions *Australian Social*
527 *Trends*, Canberra: Government of Australia 2013.
528 28 Greenland S, Pearce N. Statistical foundations for model-based adjustments. *Annual*
529 *Review of Public Health* 2015;**36**:89-108.

29 Li ZY, Chen YM, Qiu LQ et al. Prevalence, types, and malformations in congenital anomalies of the kidney and urinary tract in newborns: a retrospective hospital-based study. *Ital J Pediatr* 2019;**45**:50.

30 Chaabane S, Sheehy O, Monnier P et al. Ovarian Stimulators, Intrauterine Insemination, and Assisted Reproductive Technologies Use and the Risk of Major Congenital Malformations—The AtRISK Study. *Birth Defects Research Part B: Developmental and Reproductive Toxicology* 2016;**107**:136-147.

31 Pinborg A, Henningsen A-KA, Malchau SS et al. Congenital anomalies after assisted reproductive technology. *Fertility and Sterility* 2013;**99**:327-332.

32 Bronet F, Nogales M-C, Martínez E et al. Is there a relationship between time-lapse parameters and embryo sex? *Fertility and Sterility* 2015;**103**:396-401.e2.

33 Ding J, Yin T, Zhang Y et al. The effect of blastocyst transfer on newborn sex ratio and monozygotic twinning rate: an updated systematic review and meta-analysis. *Reproductive BioMedicine Online* 2018;**37**:292-303.

34 Zhu J, Zhuang X, Chen L et al. Effect of embryo culture media on percentage of males at birth. *Human Reproduction* 2015;**30**:1039-1045.

35 Schernhammer ES, Vitonis AF, Rich-Edwards J et al. Rotating nightshift work and the risk of endometriosis in premenopausal women. *Am J Obstet Gynecol* 2011;**205**:476 e1-8.

36 Axelsson J, Åkerstedt T, Kecklund G et al. Hormonal changes in satisfied and dissatisfied shift workers across a shift cycle. *Journal of Applied Physiology* 2003;**95**:2099-2105.

37 Peplonska B, Bukowska A, Lie JA et al. Night shift work and other determinants of estradiol, testosterone, and dehydroepiandrosterone sulfate among middle-aged nurses and midwives. *Scandinavian Journal of Work, Environment & Health* 2016:435-446.

555 **FIGURE LEGENDS**

556 **Figure 1: Construction of the dataset for analysis of the effect of maternal shift work**
557 **exposure by mode of conception.**

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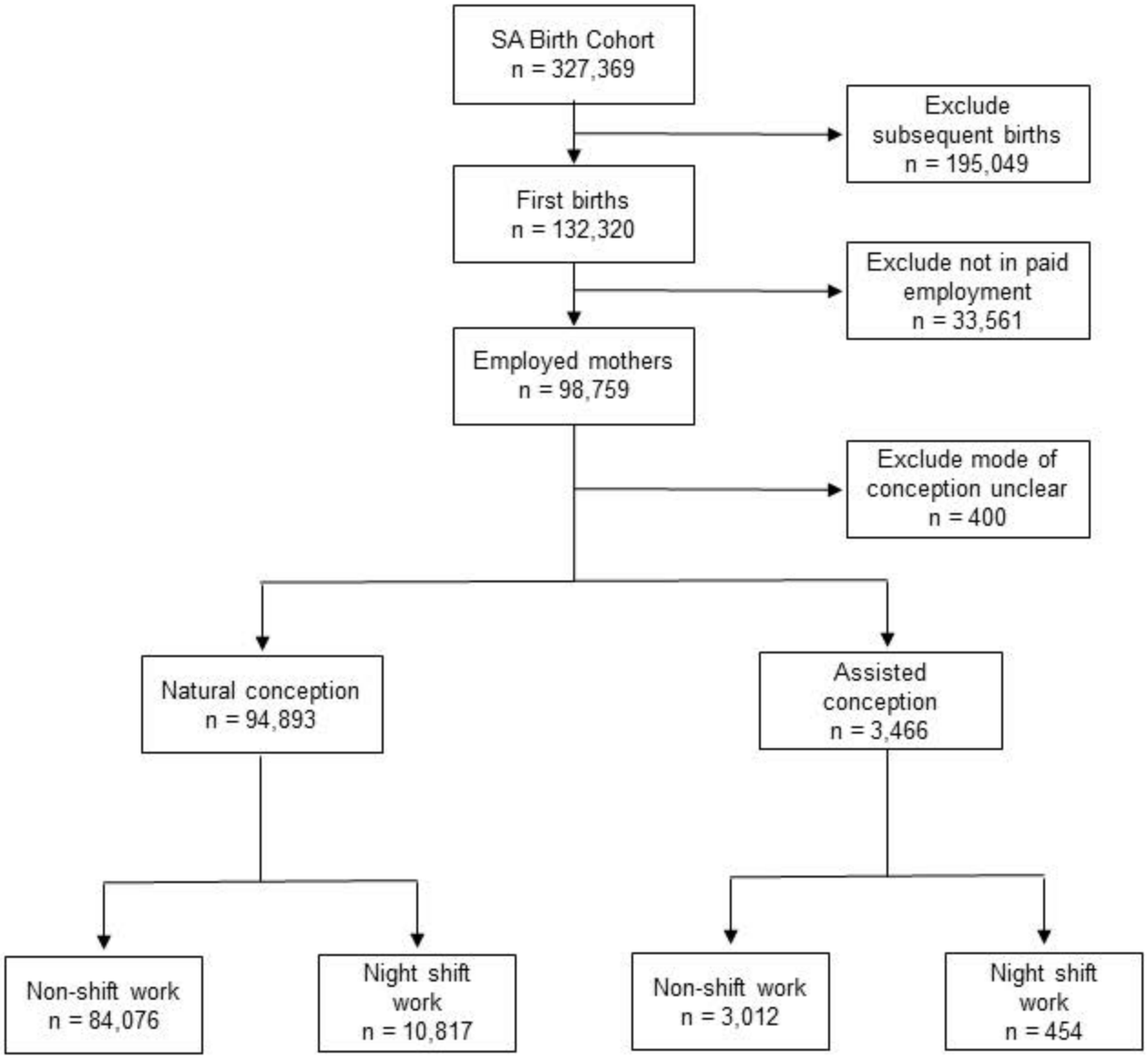
559

560 **SUPPORTING INFORMATION**

561 **Supplementary Table 1: Frequency of selected pregnancy by night shift exposure,**
562 **stratified by treatment type and multiplicity.**


SA Birth Cohort
n = 327,369
Exclude subsequent births
n = 195,049
First births
n = 132,320
Exclude not in paid employment
n = 33,561
Employed mothers
n = 98,759
Exclude mode of conception unclear
n = 400
Natural conception
n = 94,893
Assisted conception
n = 3,466
Non-shift work
n = 84,076
Night shift work
n = 10,817
Non-shift work
n = 3,012
Night shift work
n = 454

Supplementary Table 1: Frequency of selected pregnancy by night shift exposure, stratified by treatment type and multiplicity.

		Night shift work			Non-shift work		
Treatment type	**Outcome**	**Singleton n = 349**	**Twins n = 93[a] (46 sets)**	**Triplets n = 12 (4 sets)**	**Singleton n = 2127**	**Twins n = 800 (400 sets)**	**Triplets n = 72 (24 sets)**
Minimal intervention		n = 53	n = 4	n = 0	n = 349	n = 18	n = 0
	Fetal death	0	1 (25.0)		1 (0.3)	0	
	Any congenital anomalies	4 (7.5)	0		31(8.9)	0	
	Urogenital anomalies	0	0		7 (2.0)	0	
Ovulation induction only		n = 24	n = 2	n = 0	n = 177	n = 32	n = 0
	Fetal death	1 (4.2)	0		0	0	
	Any congenital anomalies	2 (8.3)	1 (50.0)		12 (6.8)	4 (12.5)	
	Urogenital anomalies	0	1 (50.0)		2 (1.1)	2 (6.3)	
Intrauterine insemination		n = 46	n = 3	n = 0	n = 290	n = 74	n = 6
	Fetal death	1 (2.2)	1 (33.3)		1 (0.3)	0	0
	Any congenital anomalies	5 (10.9)	1 (33.3)		25 (8.6)	3 (4.1)	0
	Urogenital anomalies	0	1 (33.3)		6 (2.1)	2 (2.7)	0
Gamete intrafallopian transfer		n = 24	n = 16	n = 6	n = 159	n = 92	n = 15
	Fetal death	1 (4.2)	2 (12.5)	1 (16.7)	3 (1.3)	3 (3.3)	0
	Any congenital anomalies	2 (8.3)	0	2 (33.3)	14 (8.8)	7 (7.6)	1 (6.7)
	Urogenital anomalies	1 (4.2)	0	2 (33.3)	3 (1.9)	1 (1.1)	0
In vitro fertilisation		n = 119	n = 34	n = 3	n = 615	n = 334	n = 48
	Fetal death	0	2 (5.9)	1 (33.3)	10 (1.6)	11 (3.3)	2 (4.2)

	Any congenital anomalies	13 (10.9)	5 (14.7)	0	34 (5.5)	22 (6.6)	5 (10.4)
	Urogenital anomalies	5 (4.2)	2 (5.9)	0	9 (1.5)	7 (2.1)	1 (2.1)
Intracytoplasmic sperm injection		n = 76	n = 34	n = 3	n = 501	n = 240	n = 3
	Fetal death	0	1 (2.9)	0	8 (1.6)	6 (2.5)	0
	Any congenital anomalies	10 (13.2)	3 (8.8)	0	53 (10.6)	20 (8.3)	0
	Urogenital anomalies	6 (7.9)	2 (5.9)	0	17 (3.4)	8 (3.3)	0
Donor oocyte		n = 7	n = 0	n = 0	n = 36	n = 10	n = 0
	Fetal death	0			1 (2.8)	0	
	Any congenital anomalies	0			4 (11.1)	1 (10.0)	
	Urogenital anomalies	0			1 (2.8)	0	

a. There was one twin gestation in the night shift group where one twin was lost prior to 20 weeks, and therefore did not appear in the perinatal record.