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ORIGINAL ARTICLE

Secondary sex ratio in assisted reproduction: an analysis of 1 376 454 treatment cycles performed in the UK

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STUDY QUESTION: Does ART impact the secondary sex ratio (SSR) when compared to natural conception?

SUMMARY ANSWER: IVF and ICSI as well as the stage of embryo transfer does impact the overall SSR.

WHAT IS KNOWN ALREADY: The World Health Organization quotes SSR for natural conception to range between 103 and 110 males per 100 female births.

STUDY DESIGN, SIZE, DURATION: A total of 1 376 454 ART cycles were identified, of which 1 002 698 (72.8%) cycles involved IVF or ICSI. Of these, 863 859 (85.2%) were fresh cycles and 124 654 (12.4%) were frozen cycles. Missing data were identified in 14 185 (1.4%) cycles.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All cycles recorded in the anonymized UK Human Fertilisation and Embryology Authority (HFEA) registry database between 1991 and 2016 were analysed. All singleton live births were included, and multiple births were excluded to avoid duplication.

MAIN RESULTS AND THE ROLE OF CHANCE: The overall live birth rate per cycle for all IVF and ICSI treatments was 26.2% ($n = 262\,961$), and the singleton live birth rate per cycle was 17.1% ($n = 171\,399$). The overall SSR for this study was 104.0 males per 100 female births (binomial exact 95% CI: 103.1–105.0) for all IVF and ICSI cycles performed in the UK recorded through the HFEA. This was comparable to the overall SSR for England and Wales at 105.3 males per 100 female births (95% CI: 105.2–105.4) from 1991 to 2016 obtained from the Office of National Statistics database. Male predominance was seen with conventional insemination in fresh IVF treatment cycles (SSR 110.0 males per 100 female births; 95% CI: 108.6–111.5) when compared to micro-injection in fresh ICSI treatment cycles (SSR 97.8 males per 100 female births; 95% CI: 96.5–99.2; odds ratio (OR) 1.16, 95% CI 1.12–1.19, $P < 0.0001$), as well as with blastocyst stage embryo transfers (SSR 104.8 males per 100 female births; 95% CI: 103.5–106.2) when compared to a cleavage stage embryo transfer (SSR 101.2 males per 100 female births; 95% CI: 99.3–103.1; OR 1.03, 95% CI 1.01–1.06, $P = 0.011$) for all fertilization methods.

LIMITATIONS, REASONS FOR CAUTION: The quality of the data relies on the reporting system. Furthermore, success rates through ART have improved since 1991, with an increased number of blastocyst stage embryo transfers.

WIDER IMPLICATIONS OF THE FINDINGS: This is the largest study to date evaluating the impact of ART on SSR. The results demonstrate that, overall, ART does have an impact on the SSR when assessed according to the method of fertilization (ICSI increased female births while IVF increased males). However, given the ratio of IVF to ICSI cycles at present with 60% of cycles from IVF and 40% from ICSI, the overall SSR for ART closely reflects the population SSR for, largely, natural conceptions in England and Wales.

STUDY FUNDING/COMPETING INTEREST(S): The study received no funding. C.M.B. is a member of the independent data monitoring group for a clinical endometriosis trial by ObsEva. He is on the scientific advisory board for Myovant and medical advisory board for Flo Health. He has received research grants from Bayer AG, MDNA Life Sciences, Volition Rx and Roche Diagnostics as well as from Wellbeing of Women, Medical Research Council UK, the NIH, the UK National Institute for Health Research and the European Union. He is the current Chair of

the Endometriosis Guideline Development Group for ESHRE and was a co-opted member of the Endometriosis Guideline Group by the UK National Institute for Health and Care Excellence (NICE). I.G. has received research grants from Wellbeing of Women, the European Union and Finox.

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Key words: ART / secondary sex ratio / IVF / ICSI / gender ratio / male to female births / cleavage stage transfer / blastocyst stage transfer / male subfertility

WHAT DOES THIS MEAN FOR PATIENTS?

IVF was first developed over 40 years ago. More than 250 000 babies have since been born as a result of treatment within the UK. The gender (male or female) of the child at birth is referred to as secondary sex ratio (SSR). This ratio is expressed as the number of males per 100 females at birth. The overall SSR with natural conception ranges between 103 and 110 males per 100 female births.

The Human Fertilisation and Embryology Authority (HFEA) regulates fertility treatment within the UK. This study looks at all fertility treatments leading to a live birth recorded on the HFEA database between 1991–2016. A total of 1 376 454 treatment cycles were undertaken during this time. The total number of live births has then been categorized by the sex of the child at birth and the type of treatment technique employed.

The number of female births was found to be greater than the number of male births with certain techniques, including ICSI involving the injection of sperm into each individual egg (97.8 males per 100 female births) and with the transfer of day 2–3 stage embryos, referred to as cleavage stage embryos (101.2 males per 100 female births). In contrast, an increased number of male births was seen with IVF treatments, involving the placement all collected eggs in a dish with the sperm and awaiting fertilization (110.0 males per 100 female births), and with the transfer of day 5–6 stage embryos, referred to as blastocyst stage embryos (104.8 males per 100 female births).

This is the largest study to date looking at SSR and confirms earlier suggestions of a shift in the gender balance with certain treatment types, whereby IVF treatment is shown to increase the SSR and ICSI treatment decrease the SSR, when compared to natural conception. Overall, however, the SSR for all techniques is maintained at 104.0 males per 100 female births, similar to that of natural conception, due to the current ratio of IVF to ICSI treatments. Furthermore, a shift in favour of either treatment type (IVF or ICSI) could lead to an alteration in the SSR.

Introduction

IVF was first developed over 40 years ago. More than 250 000 babies have since been born as a result of assisted reproductive treatment (ART) within the UK and millions more worldwide (HFEA, November 2016). The human sex ratio is often divided into primary (PSR) and secondary sex ratio (SSR), where the PSR refers to gender after fertilization and the SSR refers to gender at birth. A discrepancy between these two parameters can be the result of spontaneous miscarriages or terminations. These ratios are often expressed as the proportion of males or the number of males per 100 female births (Jacobsen *et al.*, 1999). Overall, the PSR is estimated to range between 107 and 170 males per 100 female births (Pergament *et al.*, 2002), while the overall SSR is 106 males per 100 female births (Grech *et al.*, 2002). The SSR for England and Wales (Fig. 1) has ranged between 103 and 107 males per 100 female births for the years 1838–2014 (Ghosh, 2019).

Previous studies have alluded to various factors that could potentially lower the SSR, such as maternal age (Rueness et al., 2012), external stressors such as war (Macmahon and Pugh, 1954), selective fetocide that predominates in certain ethnicities (Seth, 2007) and environmental influences in the form of pollution (Terrell *et al.*, 2011). While most of these discussions have been based on natural conception, recent evidence suggests that ART can also alter the SSR. A study by Dean *et al.* (2010) involving fertility clinics in Australia and New Zealand evaluated the SSR from babies born following a single embryo transfer. They demonstrated a reduction in the SSR when comparing the different fertilization methodologies of ICSI with IVF (Dean *et al.*, 2010). Furthermore, a sub-analysis of this data highlighted that the day of transfer (i.e. the stage of embryonic development during an ART cycle) also had an impact on the SSR, with a higher proportion of male births reported following a blastocyst stage transfer when compared with the transfer of a cleavage stage embryo, and this was found to be independent of the method of fertilization (Dean *et al.*, 2010).

Improvements in embryo culture media and advances with time-lapse technology have further improved the overall pregnancy rates. A retrospective, single-centre study looking at 4411 singletons born following ART demonstrated no impact on the SSR of the various culture media available with IVF treatment but did show an increase in male births with ICSI in certain culture media (Zhu et al., 2015).

However, the current evidence is conflicting with other studies demonstrating no association between ART and SSR, but they are limited by their small sample sizes (Altman and Bland, 1995; Luke *et al.*, 2009; Al-Jaroudi *et al.*, 2018). This is further reflected in studies looking at semen parameters and SSR, with even fewer patients included (Bae *et al.*, 2017; Malo *et al.*, 2017).

The inclusion of multiple births presents a compounding factor, further complicating the calculation of SSR, given that the weighting of a particular sex is dependent on the type of twin (monozygotic versus dizygotic). The increasing success rates of ART, partially attributed to extended embryo culture (Glujovsky *et al.*, 2012) and improved morphological assessment of the embryo (Harton *et al.*, 2013), increase the importance of being able to evaluate its long-term impact on society through an evaluation of gender equilibrium.

This study aims to address the imbalance of SSR in assisted reproduction. Using the wealth of the Human Fertilisation and Embryology

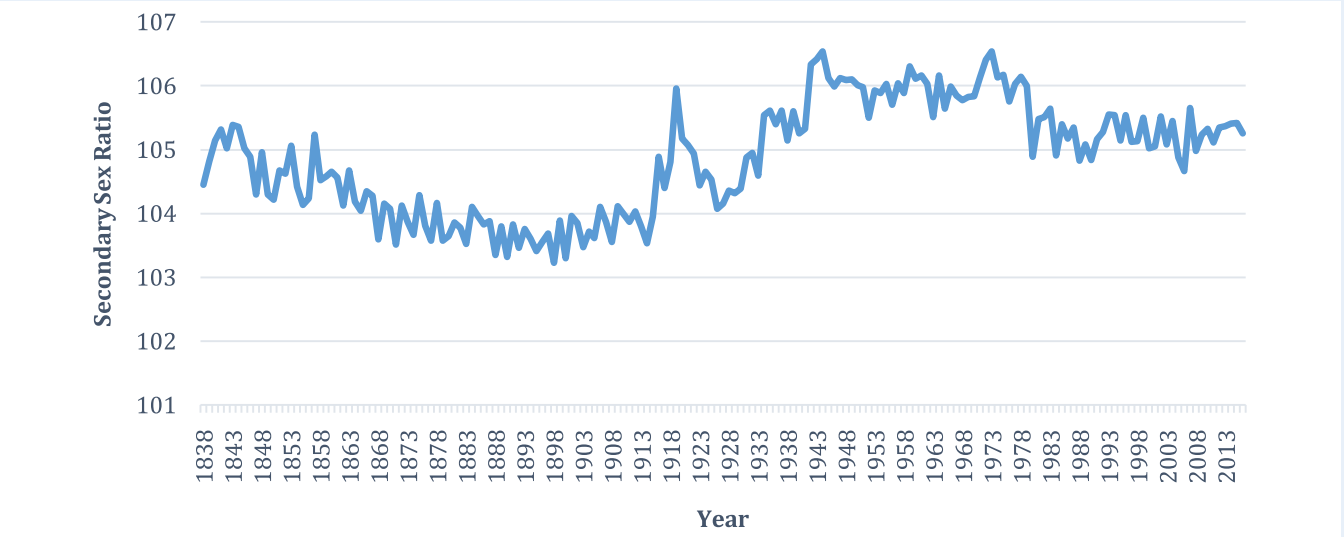

Figure 1 The SSR for England and Wales as produced by the Office of National Statistics from 1838–2016. The secondary sex ratio (SSR) on the y-axis denotes the number of males per 100 female births.

Authority (HFEA) database, this report represents the largest registry-based study involving SSR to date.

Methods

A retrospective study was carried out using the HFEA anonymized registry database analysing all singleton live births following ART between 1991 and 2016. The criteria for excluding cycles from this study were the use of IUI, IVF plus gamete intrafallopian transfer (GIFT) or IVF plus zygote intrafallopian transfer (ZIFT) and multiple pregnancies defined as more than one live birth per woman in the same pregnancy.

All UK-based licensed fertility centres are required by law to report their auditable data to the HFEA. Data from the Office of National Statistics (ONS) was obtained for all live birth SSR in England and Wales including natural and ART conceptions between 1838 and 2016 (Ghosh, 2019).

The results have been analysed in relation to maternal age (age ranges 18–34, 35–37, 38–39, 40–42, 43–44 and 45–50 years, arranged as categorical variables as per the HFEA data set, using 18–34 years as the reference category), mode of fertilization (IVF and ICSI) and stage of transfer (cleavage stage and blastocyst stage), adjusting for type of fresh treatment cycle (IVF versus ICSI), maternal age and stage of transfer.

Statistical analysis

The proportion of males was calculated with the associated exact 95% binomial CI and the SSR presented as the proportion of males per 100 female births. The data were stratified by categorical variables (female age, method of fertilization, type of cycle and stage of transfer). Binary logistic regression was conducted on the association between SSR (outcome) and each of the covariates (female age, method of fertilization and stage of transfer). Furthermore, a multivariable logistic regression was used to adjust for potential confounding variables (female age, method of fertilization and stage of transfer). A Chi-square test for association was used to compare the method of fertilization and stage of transfer. Statistical analysis was conducted using the SPSS Statistics version 22.0 (IBM, UK). A *P*-value of <0.05 was considered statistically significant.

Results

A total of 1 376 454 ART cycles were identified, of which 1 002 698 cycles incorporated IVF or ICSI (excluding cycles involving GIFT, ZIFT and IUI). Of these cycles, 863 859 (85.15%) were fresh cycles: IVF was the method of insemination in 517 402 (59.89%), and ICSI was utilized in 346 457 (40.11%) cycles. Frozen cycles accounted for 124 654 (12.43%) treatment cycles; IVF was the method of insemination in 80 995 (64.98%) and ICSI was utilized in 43 659 (35.02%) cycles. Incomplete data was identified in 14 185 (1.41%) cycles, which were excluded from the analysis.

The baseline characteristics of the included treatment cycles are described in Table I.

The overall live birth rate per included cycle was 26.2% ($n = 262\,961$), and the singleton live birth rate per cycle was 17.1% ($n = 171\,399$). The overall SSR for this study was 104.0 males per 100 female births (Binomial Exact 95% CI: 103.1–105.0). This was comparable to the overall SSR for all births in England and Wales at 105.3 males per 100 female births (95% CI: 105.2–105.4), including those conceived through ART, which accounted for ~1% of all births for the study duration (Fig. 2).

Overall, the female age was not shown to be significantly associated with the SSR. The SSR was found to be significantly different from the reference category (age 18–34 years) only for women aged 35–37 years ($n = 41\,197$ singleton live births), with a SSR of 102.6 males per 100 female births (95% CI: 100.6–104.6; odds ratio (OR) 0.96, 95% CI 0.94–0.99, $p = 0.025$), compared to the reference category 18–34 years (Tables II and III). However, it was not possible to draw

Table I Distribution of IVF and ICSI cycles according to female age groups, method of fertilization, type of treatment cycle, stage of embryo transfer and male subfertility and the corresponding live birth rate per category.

Variables	Total IVF and ICSI Cycle (n) (%)	Singleton Live Birth (n) (%)	IVF Cycle (n) (%)	ICSI Cycle (n) (%)
Age (years)				
18–34	452751 (45.7%)	90391 (20.0%)	264353 (45.1%)	188398 (46.6%)
35–37	230629 (23.3%)	41197 (17.9%)	137700 (23.5%)	92929 (23.0%)
38–39	138177 (13.9%)	19876 (14.4%)	82586 (14.1%)	55591 (13.8%)
40–42	118701 (12.0%)	12167 (10.3%)	70927 (12.1%)	47774 (11.8%)
43–44	32738 (3.3%)	2454 (7.5%)	19935 (3.4%)	12803 (3.2%)
45–50	18037 (1.8%)	2096 (11.6%)	11278 (1.9%)	6759 (1.7%)
Type of cycle				
Fresh	863859 (87.4%)	149544 (17.3%)	517402 (86.5%)	346457 (88.8%)
Frozen	124654 (12.6%)	17434 (14.0%)	80995 (13.5%)	43659 (11.2%)
Stage of Transfer				
Cleavage	221635 (35.8%)	43467 (19.6%)	105213 (35.6%)	116422 (35.9%)
Blastocyst	398078 (64.2%)	95011 (23.9%)	190084 (64.4%)	207994 (64.1%)
Male Subfertility				
Yes	448444 (44.7%)	76565 (17.1%)	185352(31.0%)	263093 (65.1%)
No	554254 (55.3%)	91616 (16.5%)	413045 (69.0%)	141209 (34.9%)

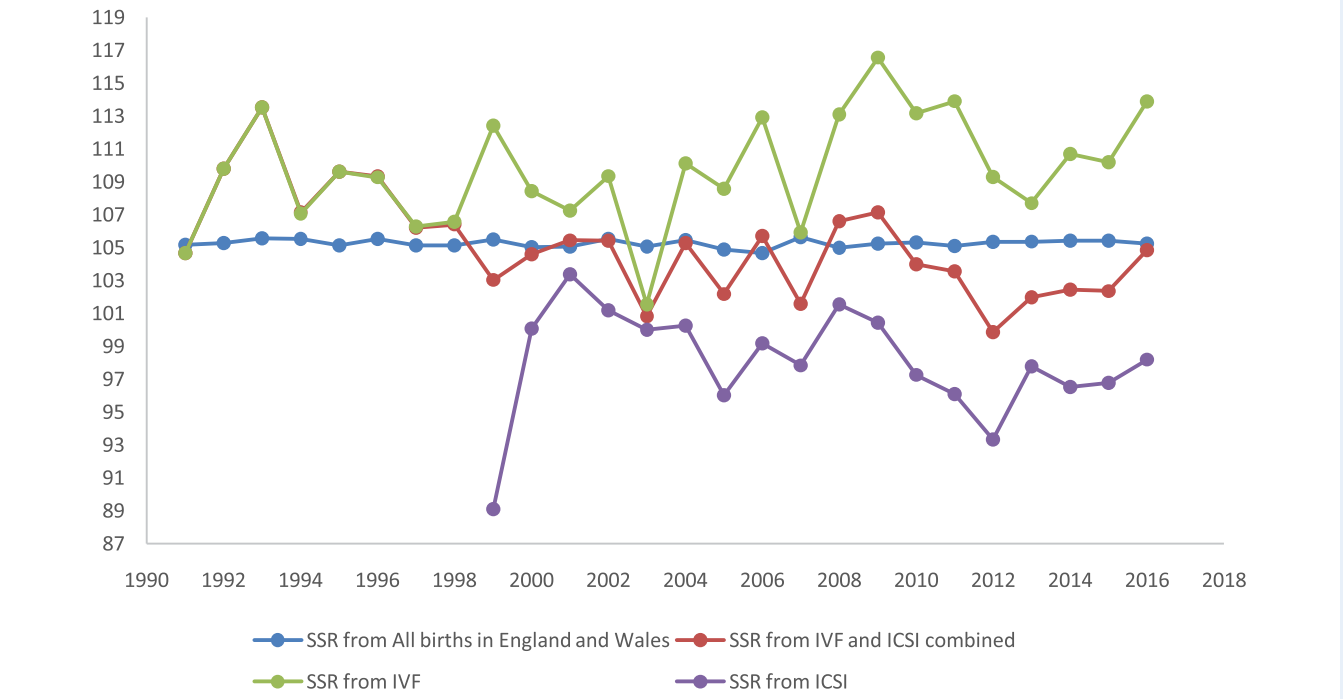

Figure 2 SSR for all births in England and Wales from 1991 to 2016 from the HFEA dataset and Office of National Statistics.

any further correlation based on the impact of female age on SSR, apart from this observed single variation.

Conventional IVF treatment cycles favoured male singleton live births, 110.0 males per 100 female births (95% CI: 108.6–111.5) when compared to ICSI, 97.8 males per 100 female births (95% CI: 96.5–99.2); OR 1.16, 95% CI 1.12–1.19, $P < 0.0001$ (Tables II and III).

A further sub-analysis showed that the SSR was not influenced by the type of cycle (fresh or frozen) (OR 0.99, 95% CI 0.93–1.05, $P = 0.708$). Univariate analysis of the impact of the method of fertilization in frozen treatment cycles on the SSR demonstrated 107.6 males per 100 female births (95% CI: 103.5–111.8) in frozen IVF treatment cycles and 96.3 males per 100 female births (95% CI: 91.9–100.9) in frozen

Table II Distribution of male offspring as a percentage and SSR according to female age groups, fertilization method, type of treatment cycle, stage of embryo transfer and male subfertility from the HFEA data set and comparative population data from the ONS.

Variables	Singleton Live Birth	Male Offspring (n)	Male Offspring (%)	Binomial Exact 95% CI	SSR*	Binomial Exact 95% CI
Age (years)						
18–34	90391	46305	51.2%	50.9–51.6	105.0	103.7–106.4
35–37	41197	20862	50.6%	50.2–51.1	102.6	100.6–104.6
38–39	19876	10081	50.7%	50.0–51.4	102.9	100.0–105.8
40–42	12167	6201	51.0%	50.1–51.9	103.9	100.0–105.8
43–44	2454	1219	49.7%	47.7–51.7	98.7	91.2–106.8
45–50	2096	1089	52.0%	49.8–54.1	108.1	99.3–117.8
Method of Fertilisation						
IVF	88291	46255	52.4%	52.1–52.7	110.0	108.6–111.5
ICSI	79890	39502	49.5%	49.1–49.8	97.8	96.5–99.2
Type of Cycle						
Fresh	149544	76299	51.0%	50.8–51.3	104.2	103.1–105.2
Frozen	17434	8840	50.7%	50.0–51.5	102.9	99.9–106.0
Frozen						
IVF	10368	5374	51.8%	50.8–52.8	107.6	103.5–111.8
ICSI	7066	3466	49.1%	47.9–50.2	96.3	91.9–100.9
Stage of Transfer						
Cleavage	43467	21861	50.3%	49.8–50.8	101.2	99.3–103.1
Blastocyst	95011	48629	51.1%	50.9–51.5	104.8	103.5–106.2
IVF Transfers						
Cleavage	20302	10536	51.9%	51.2–52.6	107.9	105.0–110.9
Blastocyst	44606	23579	52.9%	52.4–53.3	112.1	110.1–114.2
ICSI Transfers						
Cleavage	23165	11325	48.9%	48.2–49.5	95.7	93.2–98.2
Blastocyst	50405	25050	49.7%	49.3–50.1	98.8	97.1–100.5
Male Subfertility						
Yes	76565	38613	50.4%	50.1–50.8	101.7	100.3–103.2
No	91616	47144	51.5%	51.1–51.8	106.0	104.6–107.4
Database Outcomes						
ONS Population	17,366,972	8905999	51.2	51.3–51.3	105.3	105.2–105.4
HFEA IVF + ICSI	168181	85757	51.0	50.8–51.2	104.0	103.1–105.0
HFEA IVF only	88291	46255	52.4	52.1–52.7	110.0	108.6–111.5
HFEA ICSI only	79890	39502	49.5	49.1–49.8	97.8	96.5–99.2

*SSR—Secondary sex ratio defined as the number of male live births per 100 female live births.
HFEA: Human Fertilisation and Embryology Authority, ONS: Office of National Statistics

ICSI treatment cycles; OR 1.12, 95% CI 1.05–1.19, $P < 0.0001$. These findings are maintained when adjusted for stage of transfer and female age (OR 1.16, 95% CI 1.04–1.29, $P = 0.010$).

A clear predominance in favour of male births is seen with blastocyst stage embryo transfers, with an SSR of 104.8 males per 100 female births (95% CI: 103.5–106.2), in comparison to cleavage stage embryo transfers, with an SSR of 101.2 males per 100 female births (95% CI: 99.3–103.1); OR 1.03, 95% CI 1.01–1.06, p = 0.011 (Table II).

A further subgroup analysis for the method of fertilization maintained this trend, with blastocyst stage transfers resulting from both conventional IVF and ICSI treatment cycles favouring male births when compared to cleavage stage transfers (adjusted OR (aOR) 1.03, 95% CI 1.01–1.06, $P = 0.011$) (Table III). The SSR for a blastocyst stage embryo transfer resulting from conventional insemination in an IVF treatment cycle was 112.1 males per 100 female births (95% CI: 110.1–114.2; OR 1.04, 95% CI 1.00–1.08, $P = 0.033$) and 98.8 males per 100 female births (95% CI: 97.1–100.5; OR 1.03, 95% CI 1.00–1.06, $P = 0.050$) from ICSI treatment cycles, when compared to cleavage stage embryo transfers.

A total of 448 444 cycles were performed for male factor subfertility, of which 185 352 (41.3%) involved conventional IVF treatment cycles

Table III Binary logistic regression for prediction of a male birth, including the variables female age group, method of fertilization, type of treatment cycle, stage of embryo transfer and male subfertility.

Variables	Singleton Live Birth	Male Offspring (n)	SSR*	OR** (male) (95% CI)	P-value	aOR**a (male) (95% CI)	P-value
Age (years)							
18–34	90391	46305	105.0	1		1	
35–37	41197	20862	102.6	0.98 (0.95–1.00)	0.05	0.96 (0.94–0.99)	0.025
38–39	19876	10081	102.9	0.98 (0.95–1.01)	0.195	0.98 (0.93–1.01)	0.108
40–42	12167	6201	103.9	0.99 (0.95–1.03)	0.588	1.00 (0.95–1.05)	0.934
43–44	2454	1219	98.7	0.94 (0.87–1.02)	0.129	0.97 (0.86–1.08)	0.531
45–50	2096	1089	108.1	1.03 (0.94–1.12)	0.509	0.94 (0.82–1.07)	0.32
Method of Fertilisation							
IVF	88291	46255	110.0	1.13 (1.10–1.15)	<0.0001	1.16 (1.12–1.19)	<0.0001
ICSI	79890	39502	97.8	1		1	
Type of Cycle							
Fresh	149544	76299	104.2	1		1	
Frozen	17434	8840	102.9	0.99 (0.96–1.02)	0.430	0.99 (0.93–1.05)	0.708
Frozen							
IVF	10368	5374	107.6	1.12 (1.05–1.19)	<0.0001	1.16 (1.04–1.29)	0.010
ICSI	7066	3466	96.3	1		1	
Stage of Transfer							
Cleavage	43467	21861	101.2	1		1	
Blastocyst	95011	48629	104.8	1.04 (1.01–1.06)	0.002	1.03 (1.01–1.06)	0.011
IVF Transfers							
Cleavage	20302	10536	107.9	1		1	
Blastocyst	44606	23579	112.1	1.04 (1.01–1.08)	0.023	1.04 (1.00–1.08)	0.033
ICSI Transfers							
Cleavage	23165	11325	95.7	1		1	
Blastocyst	50405	25050	98.8	1.03 (1.00–1.07)	0.041	1.03 (1.00–1.06)	0.050
Male Subfertility							
Yes	76565	38613	101.7	0.96 (0.94–0.98)	<0.0001	1.03 (1.00–1.05)	0.05
No	91616	47144	106.0	1		1	

*SSR – Secondary sex ratio defined as the number of male live births per 100 female live births.
**Binary logistic regression analysis with odds ratio (OR) and 95% CI for male births by a single factor. 1 denotes the reference category.
***a*Analysis adjusted for female age, method of fertilisation and stage of transfer.

and 263 092 (58.7%) ICSI treatment cycles. The singleton live birth rate for this group was 17.1% ($n = 76\,565$). The SSR was lower for patients with male factor subfertility, at 101.7 males per 100 female births (95% CI: 100.3–103.2), compared to patients without male factor subfertility, at 106.0 males per 100 female births (95% CI: 104.6–107.4) (Table II) (OR 0.96, 95% CI 0.94–0.98, $P < 0.0001$) (Table III), suggesting an overall lower odds of a male birth in the presence of male factor subfertility. However, this trend was not maintained when adjusted for confounders (aOR 1.03, 95% CI 1.00–1.05, $P = 0.05$) (Table III), stage of transfer and method of fertilization.

Discussion

This is the largest retrospective registry-based study to date, including data from the anonymized HFEA database spanning 1991–2016, demonstrating alterations in the SSR with certain ART methodologies (Fig. 2). The effect of method of fertilization and stage of embryo transfer on the SSR was found to be statistically significant after adjusting for female age, method of fertilization and stage of transfer, thus demonstrating an independent effect of these variables on SSR.

The overall UK birth gender ratio currently stands at 105.3 males per 100 female births (95% CI: 105.2–105.4) and is considered to lie within the normal boundaries for other countries, with just over half of all infants born being male (Department of Health, 2013). A study conducted using the HFEA database and the Scottish Morbidity Record compared ART populations with naturally conceived children; the authors reported a similar SSR between the two groups at 103.7 and 103.4 males per 100 female births, respectively, suggesting no change in the SSR as a result of ART. However, a subset analysis demonstrated a lower SSR with ICSI treatment cycles, similar to the findings from this study, but they

did not reach statistical significance due to the smaller sample size (Hann *et al.*, 2018).

A number of factors have been suggested to reduce the SSR worldwide (Luke *et al.*, 2009), both biological (older age of both parents and higher maternal weight) (Nicolich *et al.*, 2000; Jacobsen, 2001) and environmental (war, earthquakes, economic distress, sex-selective termination of pregnancies, discrimination in care practices for girls [Hesketh and Xing, 2006] and toxins [smoking, pollutants, and pesticides] [Chen *et al.*, 2017]). Furthermore, during normal human development there is a trend towards sex-biased mortality, with an overall greater mortality of female foetuses during pregnancy, postulated to be secondary to disrupted expression of maternally inherited mRNA or of RNA synthesized by the embryo (Guo *et al.*, 2017). Another theory is that the paternal X chromosome retards development to such an extent that it increases the female mortality rate (Orzacka *et al.*, 2015).

Overall, IVF cycles resulted in a 16% increase in male births compared to ICSI cycles. This impact on the SSR persisted when analysed for the transfer of fresh or frozen embryos, with a 13% and 16% increase seen in male births from fresh and frozen IVF cycles, respectively, compared to ICSI treatment cycles. This suggests a greater influence of the method of fertilization (conventional IVF versus ICSI) on the SSR than the type of cycle (fresh or frozen) being undertaken.

The potential impact of ICSI on the SSR has been previously reported, leading to several hypotheses of the underlying mechanism (Graffelman *et al.*, 1999; Lobel *et al.*, 1993). One such theory is that of potential mechanical injury to the replication apparatus during microinjection of sperm into the oocyte. It has been suggested that by transecting the zona pellucida, its functional capacity can potentially be impaired by the introduction of foreign substances into the oocyte along with an alteration to the natural selection processes (Yu *et al.*, 2011; Verpoest & Tournaye, 2006). This hypothesis is supported by our finding of a lower SSR in the ICSI treatment group compared to the IVF group, as well as to the natural conception group from the ONS data (Fig. 2).

Furthermore, ICSI is thought to overcome the reduced binding ability of Y-bearing sperm to oocytes during the physiological fertilization process (Luke *et al.*, 2009), thus potentially fertilizing an oocyte with an abnormal Y-bearing sperm. It is important to bear in mind that Luke et al. (2009) demonstrated a 14% reduction in male births with ICSI in the absence of male factor subfertility, suggesting that an abnormal Y-chromosome may not be solely responsible for the differences seen in the SSR (Luke *et al.*, 2009).

In contrast to the findings in this study, whereby a male predominance is seen with blastocyst stage embryo transfers whether the method of fertilization is IVF or ICSI, Lee et al. (2016) demonstrated no difference in the SSR in euploid embryos reaching the blastocyst stage of development, determined by a complete analysis of the chromosomes through pre-implantation genetic screening (Lee *et al.*, 2016).

Blastocyst stage embryo transfers have been speculated to lead to a male predominance, secondary to their quicker growth potential due to their ability to uptake pyruvate and glucose at a higher rate compared to female embryos, thus achieving the blastocyst stage of development faster than female embryos (Pergament *et al.*, 1994; Ray *et al.*, 1995). Furthermore, male and female human embryos have different survival rates in the early stages of embryogenesis with a significantly higher primary sex ratio compared to the SSR, suggesting a poorer survival rate for male embryos overall (Luke *et al.*, 2009). Consideration should therefore be given to the development of time-lapse technology, developed to promote improved embryo selection at an earlier stage, which may inadvertently push the SSR in favour of female births while trying to avoid the impact of possible epigenetic changes with prolonged culture. Data on time-lapse technology were not available from the HFEA cohort, and therefore, this theory could not be further explored.

Earlier reports have suggested no association of the underlying cause of subfertility with the SSR (Dean *et al.*, 2010). In the present study, a sub-analysis showed a statistically significant variation in the SSR in the presence of male factor subfertility, with a 4% decrease in male births when compared to the cohort without male factor subfertility; however, this effect is not present after adjusting for potential confounders. This finding is in keeping with the abnormal Y-chromosome theory as explained by Luke *et al.* (2009). A study by Arikawa et al. (2016) demonstrated an impact on the SSR in patients undergoing IVF treatment in the presence of abnormal sperm motility when compared to those with normal sperm motility (SSR 104.08 versus 114.59, OR 0.91 95% CI 0.82–1.00) (Arikawa *et al.*, 2016).

Human ejaculation is known to contain an equal ratio of X:Y spermatozoa (Graffelman *et al.*, 1999; Viloria *et al.*, 2005; Bowman *et al.*, 1998). Studies looking at sperm swim-up techniques for sperm selection have suggested a potential impact of the method on sex selection (Jiang *et al.*, 2016). However, other studies assessing this have not reported a significant difference in the ratio of X and Y bearing spermatozoa when using a modified swim-up procedure (Yan *et al.*, 2006). It is therefore, unclear if the hypothesis that the reduction in DNA within a Y-chromosome is likely to play a role in sex selection during natural conception or standard IVF techniques (Cui, 1997).

A small study (Fedder *et al.*, 2007) assessing the gender ratio following ICSI using sperm obtained from testicular, epididymal biopsies or ejaculation found a SSR of 94 males per 100 female births, 81 males per 100 female births and 101 males per 100 female births, respectively. Fedder *et al.* (2007) also reported an SSR of 106 males per 100 female births with conventional IVF treatment. These results allude to the possibility of a reduced fertilization rate in the presence of clear male factor subfertility (Fedder *et al.*, 2007).

Limitations

The interpretation of the analysis is largely dependent on the reporting and documentation of the clinical data, despite that the number of cycles and live births analysed for each arm was powered to produce a statistically significant result. Furthermore, a previous theory of the SSR being influenced by the patient population cannot be further expanded upon because of the inability to link multiple cycles to individual patients.

Clinical practices and success rates have significantly changed since the inception of the HFEA database in 1991. Thus, inclusion of data from this time frame may impact the interpretation of the SSR. This has been accounted for by the large number of cycles included within the analysis, reducing the noise when analysed for statistical significance. Furthermore, a sub-analysis for the main treatment type and stage of embryo transfer for cycles undertaken between 2006–2016 has confirmed that the trends in SSR described have been maintained (Supplementary Table SI).

Conclusion

A number of variables have been demonstrated to influence the SSR, with a male predominance seen with conventional insemination techniques in IVF treatment cycles and blastocyst stage embryo transfers.

The overall SSR with ART when combining IVF and ICSI treatment cycles is 104.0 males per 100 female births. The SSR with natural conception ranges between 103 and 110 males per 100 female births, representative of the data from the ONS who quote an SSR of 105.3 males per 100 female births for all births within England and Wales for the same study duration (albeit acknowledging that this birth ratio does include births following ART, which account for a small proportion of the total number of births). Thus, in this study ART has been shown to clearly influence the natural gender equilibrium, with an increased number of male births following IVF treatment cycles and a decrease following ICSI treatment cycles. While the clinical relevance of this impact is still unknown, one has to bear in mind the overall impact of each method of fertilization on the alteration of the SSR and the potential route by which each intervention alters the SSR either in favour of male or female births. While the number of IVF to ICSI cycles currently being performed appears to balance the overall increase and decrease seen in the SSR, an all-ICSI policy, however, may tip the balance towards a reduction in overall male births and thus affect the gender equilibrium of pregnancies conceived through ART.

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Authors' roles

P.R.S., M.M., L.N.L, E.M., I.G. and C.M.B. were all responsible for the conception and design of the manuscript. P.R.S. was responsible for acquisition of the data. Data analysis was undertaken by P.R.S., and interpretation was by P.R.S., E.O.O. and M.M. Statistical support was provided by E.O.O. The article was drafted by P.R.S. and M.M. and critically appraised for intellectual content by E.O.O., L.N.L., E.M., I.G. and C.M.B. The final version was approved by all contributing authors P.R.S., M.M., E.O., L.N.L., E.M., I.G. and C.M.B.

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Conflict of interest

C.M.B. is a member of the independent data monitoring group for a clinical endometriosis trial by ObsEva. He is on the scientific advisory board for Myovant and medical advisory board for Flo Health. He has received research grants from Bayer AG, MDNA Life Sciences, Volition Rx and Roche Diagnostics as well as from Wellbeing of Women, Medical Research Council UK, the NIH, the UK National Institute for Health Research and the European Union. He is the current Chair of the Endometriosis Guideline Development Group for ESHRE and was a co-opted member of the Endometriosis Guideline Group by the UK National Institute for Health and Care Excellence (NICE). I.G. has received research grants from Wellbeing of Women, the European Union and Finox.

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