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.

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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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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 110 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
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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 1 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%)              | 183352 (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.
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| 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               | 16818                | 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 (n) | Male Offspring (n) | SSR* | OR** (male) (95% CI) | P-value | aOR*** (male) (95% CI) | P-value |
|----------------------------------|--------------------------|--------------------|------|----------------------|---------|------------------------|---------|
| **Age (years)**                  |                          |                    |      |                      |         |                        |         |
| 18–34                            | 90391                    | 46305              | 105.0| I                    | I       | I                      | I       |
| 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)     | 0.388   | 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 | I                    | I       | I                      | I       |
| **Type of Cycle**                |                          |                    |      |                      |         |                        |         |
| Fresh                            | 149544                   | 76299              | 104.2| I                    | I       | I                      | I       |
| 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 | I                    | I       | I                      | I       |
| **Stage of Transfer**            |                          |                    |      |                      |         |                        |         |
| Cleavage                         | 43467                    | 21861              | 101.2| I                    | I       | I                      | I       |
| 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| I                    | I       | I                      | I       |
| 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 | I                    | I       | I                      | I       |
| 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| I                    | I       | I                      | I       |

*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. I denotes the reference category.

***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: 105.2–105.4) (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 micro-injection 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 founders. 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; Viloría 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 normal 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, Voltion 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.

References

Altman DG, Bland JM. Statistics notes: absence of evidence is not evidence of absence. Br Med J 1995;311:485.
Al-Jaroudi, D., Salim, G., & Baradwan, S. (2018). Neonate female to male ratio after assisted reproduction following antagonist and agonist protocols. Medicine, 97(38), e12310. doi:10.1097/MD.0000000000012310
Anikawa M, Jwa SC, Kuwahara A, Irahara M, Saito H. Effect of semen quality on human sex ratio in vitro fertilization and intracytoplasmic sperm injection: an analysis of 27 158 singleton infants born after fresh single-embryo transfer. Fertil Steril 2016;105:897–904.
Bae J, Kim S, Chen Z, Eisenberg ML, Buck Louis GM. Human semen quality and the secondary sex ratio. Asian J Androl 2017;19:374–381.
Bowman M, De Boer K, Cullinan R, Catt J, Jansen R. Do alterations in the sex ratio occur at fertilization? A case report using fluorescent in situ hybridization. J Assist Reprod Genet 1998;15:320–322.
Chen M, Du J, Zhao J, Lv H, Wang Y, Chen XJ, Zhang J, Hu L, Jin G, Shen H et al. The sex ratio of singleton and twin delivery offspring in assisted reproductive technology in China. Nat Sci Rep 2017;7:1–8.
Cui KH. Size differences between human X and Y spermatozoa and prefertilization diagnosis. Mol Hum Reprod 1997;3:61–67.
Dean JH, Chapman MG, Sullivan EA. The effect on human sex ratio at birth by assisted reproductive technology (ART) procedures - an assessment of babies born following single embryo transfers, Australia and New Zealand, 2002-2006. BJOG 2010;117:1628–1634.
Department of Health. Birth Ratios in the United Kingdom. A report on gender ratios at birth in the UK, May 2013.
Fedder J, Gabrielsen A, Humaider P, Erb K, Ernst E, Loft A. Malformation rate and sex ratio in 412 children conceived with epididymal or testicular sperm. Hum Reprod 2007;22:1080–1085.
Ghosh K. Office of National Statistics. 2019. https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/datasets/birthcharacteristicsinenglandandwales (10 June 2019, date last accessed)
Guo, L., Zhang, Q., Ma, X., Wang, J., & Liang, T. (2017). miRNA and mRNA expression analysis reveals potential sex-biased miRNA expression. Sci Rep, 7, 39812. doi:10.1038/srep39812.
Grech V, Savona-Ventura C, Vassallo-Agius P. Unexplained differences in sex ratios at birth in Europe and North America. BMJ 2002;324:7344.
Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev 2012;7:1465–1458.
Graffelman J, Fugger EF, Keyvanfar K, Schulman JD. Human live birth and sperm-sex ratios compared. Hum Reprod 1999;14:2917–2920.
Hann, M., Roberts, S. A., D’Souza, S. W., Clayton, P., Macklon, N., & Brison, D. R.. The growth of assisted reproductive treatment-conceived children from birth to 5 years: a national cohort study. BMC Med 2018;16:224.
Harton GL, Munne S, Surrey M, Grino J, Kaplan B, McCulloh DH, Griffin DK, Wells D, PGD Practitioners Group. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril 2013;100:1695–1703.
The influence of ART on secondary sex ratio

Hesketh T, Xing ZW. Abnormal sex ratios in human populations: causes and consequences. Proc Natl Acad Sci U S A 2006;103:13271–13275.

HFEA. Fertility treatment 2014-2016: Trends and figures. https://www.hfea.gov.uk/media/2563/hfea-fertility-trends-and-figures-2017-v2.pdf (5 June 2018, date last accessed)

Jacobsen R, Møller H, Mouritsen A. Natural variation in the human sex ratio. Hum Reprod 1999;14:3120–3125.

Jacobsen R. Parental ages and secondary sex ratio. Hum Reprod 2001;16:2244.

Jiang M, Wen Y, Yang W, He W, Zhang B, Cai L. Sperm processing affected ratio of X- and Y-bearing sperm. Int J Clin Exp Pathol 2016;9:8550–8554.

Lee H, McCulloh DH, Macanas E, McCaffrey C, Grifo J. Gender ratio and live birth after preimplantation genetic screening (PGS). Fertil Steril 2016;106:e161–e162.

Lobel SM, Pomponio RJ, Mutter GL. The sex ratio of normal and manipulated human sperm quantitated by the polymerase chain reaction. Fertil Steril 1993;59:387–392.

Luke B, Brown MB, Grainger DA, Baker VL, Ginsburg E, Stern JE et al. The sex ratio of singleton offspring in assisted-conception pregnancies. Fertil Steril 2009;92:1579–1585.

Macmahon B, Pugh TF. Sex ratio of white births in the United States during the Second World War. Am J Hum Genet 1954;6:284–292.

Malo AF, Martinez-Pastor F, Garcia-Gonzalez F, Garde J, Ballou JD, Lacy RC. A father effect explains sex-ratio bias. Proc R Soc B Biol Sci 2017;284:1–7.

Nicolich MJ, Huebner WW, Schnatter AR. Influence of parental and biological factors on the male birth fraction in the United States: an analysis of birth certificate data from 1964 through 1988. Fertil Steril 2000;73:487–492.

Orzacka SH, Stubblefielda W, Akmaevb VR, Collsc P, Munnéc S, Schollb T, Steinsaltdz D, Zuckermane JE. The human sex ratio from conception to birth. PNAS 2015;E2102–E2111.

Pergament E, Fiddler M, Cho N, Johnson D, Holmgren WJ. Sexual differentiation and preimplantation cell growth. Hum Reprod 1994;9:1730–1732.

Pergament E, Toydemir PB, Fiddler M. Sex ratio: a biological perspective of ‘Sex and the City’. Reprod Biomed Online 2002;5:43–46.

Ray PF, Conaghan J, Winston RM, Handyside AH. Increased number of cells and metabolic activity in male human preimplantation embryos following in vitro fertilization. J Reprod Fertil 1995;104:165–171.

Rueness J, Vatten L, Eskild A. The human sex ratio: effects of maternal age. Hum Reprod 2012;27:283–287.

Seth S. Sex selective feticide in India. J Assist Reprod Genet 2007;24:153–154.

Terrell ML, Hartnett KP, Marcus M. Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. Emerg Health Threats J 2011;4:7109.

Viloria T, Rubio MC, Rodriguez L, Calderon G, Mercader A, Mateu E, Meseguer M, Remohi J, Pellicer A. Smoking habits of parents and male: female ratio in spermatozoa and preimplantation embryos. Hum Reprod 2005;20:2517–2522.

Willem Verpoest & Herman Tournaye (2006) ICSI: hype or hazard?. Hum Fertil, 9:81–92, DOI: 10.1080/14647270500422158

Yan J, Feng HL, Chen ZJ, Hu J, Gao X, Qin Y. Influence of swim-up time on the ratio of X- and Y-bearing spermatozoa. Eur J Obstet Gynecol Reprod Biol 2006;129:150–154.

Yu Y, Zhao C, Lv Z, Chen W, Tong M, Guo X, Wang L, Liu J, Zhou Z, Zhu H et al. Microinjection manipulation resulted in the increased apoptosis of spermatocytes in testes from intracytoplasmic sperm injection (ICSI) derived mice. PLoS One 2011;6:e22172.

Zhu J, Zhuang X, Chen L, Liu P, Qiao J. Effect of embryo culture media on percentage of males at birth. Hum Reprod 2015;30:1039–1045.