The human sex ratio from conception to birth

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Edited by James R. Carey, University of California, Davis, CA, and accepted by the Editorial Board February 24, 2015 (received for review August 27, 2014)

We describe the trajectory of the human sex ratio from conception to birth by analyzing data from (*i*) 3- to 6-d-old embryos, (*ii*) induced abortions, (*iii*) chorionic villus sampling, (*iv*) amniocentesis, and (*v*) fetal deaths and live births. Our dataset is the most comprehensive and largest ever assembled to estimate the sex ratio at conception and the sex ratio trajectory and is the first, to our knowledge, to include all of these types of data. Our estimate of the sex ratio at conception is 0.5 (proportion male), which contradicts the common claim that the sex ratio at conception is male-biased. The sex ratio among abnormal embryos is male-biased, and the sex ratio among normal embryos is female-biased. These biases are associated with the abnormal/normal state of the sex chromosomes and of chromosomes 15 and 17. The sex ratio may decrease in the first week or so after conception (due to excess male mortality); it then increases for at least 10–15 wk (due to excess female mortality), levels off after ∼20 wk, and declines slowly from 28 to 35 wk (due to excess male mortality). Total female mortality during pregnancy exceeds total male mortality. The unbiased sex ratio at conception, the increase in the sex ratio during the first trimester, and total mortality during pregnancy being greater for females are fundamental insights into early human development.

demography | development | evolution | genetics | sex ratio

The sex ratio at conception in humans is unknown, despite hundreds of years of speculation and research. Investigations of the sex ratio date back at least as far as Graunt (1) who described a net excess of male births (2). By the late 1800s, it was clear that more males than females die during later pregnancy (3). Beyond these facts, the demographic and genetic dynamics of the sex ratio from conception to birth are poorly resolved.

The claim that the conception or primary sex ratio (PSR) is more male-biased than the birth sex ratio appears often in textbooks (4, 5) and in the scientific literature (e.g., refs. 6–11), usually with little or no description of evidence. Estimates of the PSR in these studies are typically 0.56 (proportion males) or greater. Many fewer researchers have claimed that the PSR is unbiased or slightly male-biased (12–16). A handful of researchers has claimed or implied that the PSR is female-biased (17–19) or claimed that the PSR cannot be estimated due to lack of appropriate data and/or methodological problems (20–22).

Previous estimates of the PSR have no meaningful basis in data from the time of conception (or within at least a month of it). At best, the PSR has been estimated via backward extrapolation from data on induced or spontaneous abortions, fetal deaths, or live births; most of the non–live-birth data stems from the second or third trimester of pregnancy. In addition, even if one ignores the fallibility of extrapolation, biased estimates of the PSR based on spontaneous abortions and fetal deaths have usually been regarded as arising from unbiased samples of a population of embryos or fetuses having a biased PSR. The alternative possibility that the estimates arise from biased samples of a population having an unbiased PSR has received little attention. The most likely source of bias is the differential tendency of the two sexes to die during pregnancy, which has long been recognized (see above), although its implications for the estimation of the PSR have usually been ignored.

Here, we estimate the trajectory of the sex ratio from conception to birth by analyzing 3- to 6-d-old embryos derived from assisted reproductive technology (ART) procedures, induced abortions, fetuses that have undergone chorionic villus sampling (CVS) or amniocentesis, and US census records of fetal deaths and live births. Our assemblage of data is the most comprehensive and largest ever assembled to estimate the PSR and the sex ratio trajectory and is the first, to our knowledge, to include all of these types of data.

Materials and Methods

We measured gestation time as elapsed time since conception (syngamy) or conception age (CA). CA estimates were inferred from the date of the last menstrual period (LMP) or the clinical estimate (based on an ultrasound scan or the assessment of the birth attendant) by subtracting 2 wk from the original estimate. This approximation captures the central tendency of the distribution of days since the date of conception; the modal time is 15 d, and more than 50% of conceptions are estimated to occur between 12 and 16 d after LMP (23).

We defined the cohort sex ratio (CSR) at a given CA as the sex ratio of the cohort of embryos (fetuses) inside mothers. CSR is directly calculated from amniocentesis, CVS, and induced-abortion data and inferred from ART and fetal-death and live-birth data. By definition, the PSR is equal to the CSR at conception. We further defined the abnormal CSR and the normal CSR as the cohort sex ratio of embryos (fetuses) that were karyotypically abnormal and karyotypically normal, respectively.

We analyzed five kinds of data.

Three- to 6-d-Old Embryos. We used FISH or array comparative genomic hybridization (aCGH) to karyotype embryos. See ref. 24 for an overview of FISH and refs. 25 and 26 for reviews of its use for karyotypic assessment. FISH may

Significance

The human sex ratio has long interested cell biologists, developmental biologists, demographers, epidemiologists, evolutionary biologists, gynecologists, and statisticians. Nonetheless, the trajectory of the human sex ratio from conception to birth has been poorly characterized. We present the most comprehensive analysis of this trajectory ever done. Our dataset is the largest ever assembled to estimate the sex ratio at conception and is the first, to our knowledge, to include data from 3- to- 6-d-old embryos, induced abortions, chorionic villus sampling, amniocentesis, and fetal deaths and live births. Our results indicate that the sex ratio at conception is unbiased, the proportion of males increases during the first trimester, and total female mortality during pregnancy exceeds total male mortality; these are fundamental insights into early human development.

Author contributions: S.H.O., J.W.S., S.M., and J.E.Z. designed research; S.H.O., J.W.S., P.C., S.M., and D.S. performed research; V.R.A., P.C., S.M., and T.S. contributed new reagents/analytic tools; S.H.O. and D.S. analyzed data; and S.H.O. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. J.R.C. is a guest editor invited by the Editorial Board.

Freely available online through the PNAS open access option.

See Commentary on page 4839.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1416546112/-/DCSupplemental.

overestimate the incidence of aneuploidy (27). There is no indication that this would influence sex ratio estimates. Chromosomes X, Y, 8, 9, 13, 14, 15, 16, 17, 18, 20, 21, and 22 were scored. The number of chromosomes scored for a given embryo ranged from 2 (X and Y) to 13. The FISH probes are shown in the *SI Text*. Embryos analyzed by FISH were at least 3 d old and were at the blastomere stage; almost all were 3 d old. See refs. 28 and 29 for an overview of aCGH. All chromosomes were scored. Embryos analyzed by aCGH were between 3 and 6 d old; ~30% were 3 d old (blastomere stage), with most of the remainder being 5 d old (blastocyst stage). FISH and aCGH produce functionally equivalent screens of karyotypic abnormality (30); further cross-validation is needed.

Most embryos had one cell analyzed; results for embryos with multiple cells analyzed are aggregated over the cells. Embryos analyzed by FISH included all submitted for analysis, even if developmentally arrested by day 3. Embryos analyzed by aCGH included only those that were not arrested at the time of sampling (days 3–6).

An embryo was scored as a male if it had a Y chromosome in at least one cell and as a female if it had no Y chromosome and at least two X chromosomes. An embryo was scored as normal if cells were identically XX or XY and had exactly two copies of each autosome scored. Other sexable karyotypes were scored as abnormal. There were 139,704 sexable embryos (94,535 FISH and 45,169 aCGH).

Induced Abortions. To our knowledge, there are only 41 studies of the sex of fetuses from induced abortion (*SI Text*); these data have never before been assembled and analyzed. It is almost certain that all fetuses were naturally conceived (most analyses were published before 1978, when ART was introduced) and virtually all were sampled randomly with respect to fetal health and sex. The methods used to assign sex were histology (1 study), karyotype (20 studies), morphology (3 studies), and sex chromatin (17 studies). Thirty-nine studies specify trimester for each fetus; of these, 12 studies provide data allowing a CSR estimate for trimester 1 and for trimester 2. Twenty-four studies specify gestational age in weeks.

CVS. The procedures used to process and assess each sample are shown in *SI Text*. The use of CVS is reviewed in refs. 31 and 32.

The CVS data provided estimates of the CSR from 6 to 25 wk CA. In our analysis of the relationship between CSR and CA, we used data from 6 to 12 wk (97% of the sample) to avoid possible overrepresentation of troubled pregnancies. In almost all cases, CA estimates were based on the LMP.

Amniocentesis. The procedures used to process and assess each sample were identical to those for CVS (*SI Text*). The use of amniocentesis is reviewed in refs. 31 and 32.

The amniocentesis data provided estimates of the CSR from 10 to 39 wk CA. In our analysis of the relationship between CSR and CA, we used data from 10 to 20 wk (96% of the sample) to avoid possible overrepresentation of troubled pregnancies and because the cohort of fetuses is increasingly influenced by birth after 24 wk (so that mortality is not the sole influence on the CSR). In almost all cases, CA estimates were based on the LMP.

Our ART, CVS, and amniocentesis data were based on similar criteria for scoring karyotypes and therefore provide comparable insights into the CSR from the beginning of pregnancy to the end.

Fetal Deaths and Live Births. We created a dataset containing sex and CA for all US fetal deaths and live births for 1995–2004 using data from www.cdc.gov/nchs/data_access/Vitalstatsonline.htm. Reporting is poor before 18 wk CA, and it is nearly complete only after 25 wk (33). We included CA estimates derived from the LMP and from the clinical estimate. We omitted records with imputed sex or gestational age (*SI Text*).

Statistical Approach. We estimated sex ratios using mixed-effect analyses (34) or fixed-effect analyses on the logit scale. All model comparisons involved nested models. We provide two ways of assessing a given model comparison. First, we present the absolute difference (ΔAIC) between the model with lowest Akaike information criterion (AIC) value and the other model(s). A ΔAIC value of 2 or more is often taken to indicate that two models differ in their level of support (35, pp. 70–71). Second, we present the Akaike weight for each model. The evidence ratio (ER) for a pair of models is the ratio of their weights (larger/smaller), which is equivalent to the ratio of their model likelihoods. An ER between 100 and 1,000 denotes strong support for the model with the larger weight (36). [An Akaike weight is also controversially interpreted as an approximate Bayesian posterior probability that the model is true given the assumption that the true model is contained in the set of models considered (37–39).] One can also assume that the simplest model among those considered is a true null hypothesis and estimate the probability that a ΔAIC value could have arisen via random sampling (40–43). Critical values of ΔAIC depend on the difference, k, in the number of model parameters between the null and alternative hypotheses (43). For example, for k = 5 (all model comparisons in Tables 1–4 and Tables S3–S5), critical values for ΔAIC are 1.07 ($\alpha = 0.05$), 5.09 ($\alpha = 0.01$), 6.75 ($\alpha = 0.005$), and 10.52 ($\alpha = 0.001$). Model comparisons here differ in k, but the critical value of ΔAIC for $\alpha = 0.05$ is at most 1.84 and for $\alpha = 0.01$ it is at most 5.34. In all tables, *N* denotes sample size.

Results

Analysis of ART Data. We assigned random effects to women and to procedures within women and treated karyotypic state as a factor.

We first estimated the PSR. For all embryos (Any) in Table 1, the CSR estimate of 0.502 (95% CI: 0.499–0.505) suggests that the PSR is unbiased or slightly male-biased. This estimate derives from the largest amount of data ever assembled from a known time close to conception; an estimate closer to conception is likely impossible.

The model stratified with karyotypic state (Abnormal and Normal) had substantially more support than a model without stratification (Any); the ER for the stratified and unstratified models is greater than 1,000 (≥0.999/<0.001). The abnormal CSR estimate is 0.508 (95% CI: 0.505–0.512), and the normal CSR estimate is 0.493 (95% CI: 0.488–0.497). These estimates suggest that very early development is more hazardous for males than for females. Nature's filter against abnormalities such as aneuploidy must be similar to our filter because the frequency of such abnormalities among newborns is 1% at most. This frequency implies that most abnormalities cause embryonic death [although embryos may self-correct (44)]; the timing of mortality may be such that the CSR is temporarily female-biased soon after conception.

We assessed if CSR estimates depended on whether one cell or more than one cell was scored (Table 2) because it is possible that mosaic embryos were falsely scored as normal because abnormal cells were not scored; only FISH data were analyzed (few aCGH analyses involved more than one cell). Most had one cell (90,580 embryos) or two cells (2,567 embryos) scored. The CSR estimates based on one cell qualitatively match those based on more than one cell. When one cell was scored, the stratified model had greater support. When multiple cells were scored, the nonstratified and stratified models had similar support; this is likely due to a small sample size. These results suggest that the false scoring of abnormal embryos as normal has little influence on our observation that the normal CSR is female-biased (Table 1).

We assessed the association of each target chromosome and the CSR in two ways. In the first, the embryo could be normal or abnormal for any other chromosome (Table 3); FISH and aCGH data are presented separately. Estimates of the CSR for FISH and aCGH based on any chromosome are 0.503 (95% CI: 0.500–0.507, $n = 94,535$) and 0.500 (95% CI: 0.495–0.505, $n = 45,169$), respectively. The CSR estimate "all" is ~0.500 for each target chromosome assayed by FISH. This similarity suggests that the embryos chosen for analysis of a given target chromosome were chosen randomly from the assemblage. (There is only one CSR

Table 1. Mixed-effect analyses of the association between the karyotypic state of all ART embryos and the CSR

Scoring	Embryos	CSR	*N*	ΔAIC	Akaike weight
Any	All	0.502	139,704	22.870	<0.001
	Abnormal	0.508	84,881	0	>0.999
	Normal	0.493	54,823		

Scoring denotes the chromosomes used to assess karyotypic state. Any denotes assessment based on any number of chromosomes scored (between 2 and 23).

Table 2. Mixed-effect analyses of the association between the karyotypic state of ART embryos analyzed by FISH and the CSR when one cell was scored and when more than one cell was scored

Number of cells scored	Embryos	CSR	*N*	ΔAIC	Akaike weight
1	All	0.503	90,580	27.107	<0.001
	Abnormal	0.511	56,354	0	>0.999
	Normal	0.491	34,226		
>1	All	0.502	3,955	0	0.731
	Abnormal	0.513	3,170	2.374	0.269
	Normal	0.458	785		

estimate "all" for the aCGH analyses because the same embryos provided all of the target chromosome estimates.)

As noted, the FISH sample included arrested and nonarrested embryos and the aCGH sample contained only nonarrested embryos (most had undergone blastocyst formation). Comparison of the two samples provides insight into the early association between chromosome abnormality and the attainment of a critical developmental milestone.

For the FISH sample, there was greater support for the nonstratified model for all but three of the chromosomes, which suggests that there is no sex bias in the expression of abnormality for most chromosomes. For XY, 15, and 17, there was greater support for the stratified model. The ER is ∼140 for chromosome 17 and is >1,000 for XY and for chromosome 15. Thus, there is strong to very strong support for a sex bias in the abnormality of these chromosomes. For these cases, the abnormal CSR estimate is male-biased and the normal CSR estimate is female-biased. Note that the abnormal CSR estimate (0.589) for the embryos with abnormal sex chromosomes (XY) is biased upward because XO embryos are not included (*Discussion*).

For the aCGH sample, there was greater support for the nonstratified model for all but 4 of the 23 chromosomes, which suggests that there is no sex bias in the expression of abnormality for most chromosomes. For chromosomes 5 and 22, there was marginally greater support for the stratified model. The ER is ∼2 for both. For chromosomes XY and 7, there is moderate to very strong support for a sex bias of abnormality. The ER is >1,000 for XY and is ∼9 for chromosome 7. As noted above, the abnormal CSR estimate (0.840) for the embryos with abnormal sex chromosomes is biased upward. The abnormal CSR estimate for chromosome 7 is female-biased.

The male bias among FISH embryos abnormal for chromosome 15 (0.518) and for 17 (0.517) and the female bias among abnormal aCGH embryos (15: 0.490; 17: 0.480) are consistent with excess death of male embryos before the time of blastocyst formation. We lack data on chromosome 7 among FISH embryos, but the support for the stratified model among aCGH embryos suggests that this chromosome may also play an important role in blastocyst formation.

In the second way we assessed the association of each target chromosome and the CSR, all scored chromosomes were normal except the target chromosome, which could be normal or abnormal (*SI Text*). This analysis allowed us to assess whether the association between the state of a target chromosome and the CSR was a consequence of the target chromosome by itself or of an ensemble of chromosomes (in which only the target chromosome has a known state). There are relatively few embryos that are abnormal for just one chromosome. Only the analysis for XY suggests substantially greater support for the stratified model. For chromosome 15, the abnormal CSR estimate is female-biased compared with the normal CSR estimate, which is reversed compared with when other chromosomes could be normal or abnormal; reasons for this other than reduced sample size are unclear. For chromosome 17, the abnormal CSR estimate is male-biased compared with the normal CSF estimate, which is the same as when other chromosomes were normal or abnormal.

Taken together, these results indicate that abnormalities occur more frequently in male embryos than in female embryos and suggest that the female bias of the normal CSR estimate (0.493; Table 1) is associated with abnormality of just a few autosomes. However, the role of each of these autosomes by itself is ambiguous. See *Discussion* for the possible cause of the association of chromosome 15 and the abnormal CSR. The decrease in the abnormal CSR estimate pre- and postarrest (Table 3; Any: 0.511 vs. 0.502) is consistent with embryonic mortality before blastocyst formation being male-biased. The normal CSR estimate is female-biased, which implies that the CSR may temporarily become female-biased due to the death of karyotypically abnormal embryos.

There were differences among chromosomes in frequency of abnormalities. The frequency of karyotypic abnormality is greater in the FISH sample compared with the aCGH sample, the likely reason being that most abnormalities are incompatible with continuing development. The average frequency of abnormality for FISH is 25.39% (low: 17.22% for XY, high: 31.31% for chromosome 22), and for CGH, it is 6.94% (low: 4.15% for XY, high: 11.48% for chromosome 16). There is significant heterogeneity among chromosomes for frequency of abnormality (FISH: χ^2 = 7,679.748, 11 df, $P \ll 0.001$; CGH: χ^2 = 6,193.179, 22 df, $P \ll 0.001$). (There is also significant heterogeneity when the sex chromosomes are omitted; as noted, their frequency of abnormality is underestimated.) These statistical tests have the probably incorrect assumption that abnormality for a chromosome occurs independently of abnormality for other chromosomes.

Additional analyses of the association between karyotype and the CSR are shown in *SI Text* (blastomere aCGH data vs. blastocyst aCGH data and blastomere FISH data vs. blastocyst aCGH data).

We analyzed maternal age (MA) as a metric predictor of the CSR (Table 4). The model without age has strong support (ER ∼ 33), which suggests that there is no association between the CSR and maternal age; most studies indicate that maternal age has little or no influence on the sex ratio at birth (45–46).

Analysis of limited data (n = 819) suggested that there is no association between mother's race and the CSR. We compared an overall model, a model stratified between black and nonblack mothers, and a model stratified between white and nonwhite mothers. The overall model had substantially greater support than either stratified model.

Analysis of Induced-Abortion Data. We assessed the effect of trimester on the CSR by using a mixed-effect analysis in which random effects were assigned to each study (Table 5); we analyzed only the data from the 12 studies that each provided a first and second trimester estimate. We did not distinguish between diagnostic methods or between abnormal and normal sex ratios because karyotypic information for aborted fetuses is limited. The stratified model had greater support (ER ∼ 10.6). The associated estimates suggest that the CSR increases with trimester (first: 0.511 vs. second: 0.559). This increase is consistent with greater net female mortality during the first and second trimesters (see below).

We also assessed the relationship between the CSR and CA by using a mixed-effect logistic regression analysis in which random effects were assigned to each study. The sole study based on histology was omitted because it contained fetuses of a single age. Fourteen of the remaining 23 studies present a several-week range of CA for some or all fetuses. We fit separate models for the early CA estimates and for the late estimates. A model with no influence of CA as a metric predictor had the most support. In keeping with the increased CSR estimate in the second trimester compared with the first trimester (see above), we present the CSR estimates based on the early CA estimates (Table 6; the CSR estimates based on late CA estimates are qualitative identical). The model with most support was diagnostic method specific (ER ∼ 39). We focus on the chromatin and karyotype

Table 3. Mixed-effect analyses of the association between the overall state of the embryo (Any) or the state of individual chromosomes and the CSR

		FISH				aCGH			
Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight	CSR	*N*	ΔAIC	Akaike weight
Any	All	0.503	94,535	31.275	<0.001	0.500	45,169	0	0.953
	Abnormal	0.511	59,524	0	>0.999	0.502	24,357	6.004	0.047
	Normal	0.490	35,011			0.498	19,812		
XY	All	0.503	94,535	533.156	<0.001	0.500	45,169	850.311	<0.001
	Abnormal	0.589	16,282	0	>0.999	0.840	1,874	0	>0.999
	Normal	0.486	78,253			0.486	43,295		
1	All	—	—	—	—	0.500	45,169	0	0.942
	Abnormal	—	—	—	—	0.481	2,972	5.571	0.058
	Normal	—	—	—	—	0.502	42,197		
2	All	—	—	—	—	0.500	45,169	0	0.784
	Abnormal	—	—	—	—	0.478	2,856	2.579	0.216
	Normal	—	—	—	—	0.502	42,313		
3	All	—	—	—	—	0.500	45,169	0	0.982
	Abnormal	—	—	—	—	0.486	2,255	7.898	0.018
	Normal	—	—	—	—	0.501	42,914		
4	All	—	—	—	—	0.500	45,169	0	0.948
	Abnormal	—	—	—	—	0.484	2,459	5.704	0.052
	Normal	—	—	—	—	0.501	42,710		
5	All	—	—	—	—	0.500	45,169	1.460	0.325
	Abnormal	—	—	—	—	0.468	2,547	0	0.675
	Normal	—	—	—	—	0.502	42,622		
6	All	—	—	—	—	0.500	45,169	0	0.959
	Abnormal	—	—	—	—	0.483	2,365	6.300	0.041
	Normal	—	—	—	—	0.501	42,804		
7	All	—	—	—	—	0.500	45169	4.400	0.100
	Abnormal	—	—	—	—	0.466	2,637	0	0.900
	Normal	—	—	—	—	0.502	42,532		
8	All	0.505	22,113	0	0.984	0.500	45,169	0	0.983
	Abnormal	0.503	4,119	8.274	0.016	0.488	2,638	8.102	0.017
	Normal	0.506	17,994			0.501	42,531		
9	All	0.524	3,678	0	0.947	0.500	45,169	0	0.845
	Abnormal	0.516	655	5.780	0.053	0.478	3,010	3.394	0.155
	Normal	0.526	3,023			0.502	42,159		
10	All	—	—	—	—	0.500	45,169	0	0.951
	Abnormal	—	—	—	—	0.481	2,683	5.930	0.049
	Normal	—	—	—	—	0.501	42,486		
11	All	—	—	—	—	0.500	45,169	0	0.962
	Abnormal	—	—	—	—	0.484	2,748	6.438	0.038
	Normal	—	—	—	—	0.501	42,421		
12	All	—	—	—	—	0.500	45,169	0	0.978
	Abnormal	—	—	—	—	0.486	2,360	7.583	0.022
	Normal	—	—	—	—	0.501	42,809		
13	All	0.503	89,263	0	0.976	0.500	45,169	0	0.936
	Abnormal	0.505	23,598	12.075	0.024	0.482	3,133	5.361	0.064
	Normal	0.503	65,665			0.502	42,036		
14	All	0.503	18,378	0	0.992	0.500	45,169	0	0.936
	Abnormal	0.500	4,727	9.542	0.008	0.485	3,078	5.366	0.064
	Normal	0.504	13,651			0.501	42,091		
15	All	0.500	78,437	42.555	<0.001	0.500	45,169	0	0.963
	Abnormal	0.518	24,120	0	>0.999	0.490	4,209	6.512	0.037
	Normal	0.492	54,317			0.501	40,960		
16	All	0.504	79,589	0	0.881	0.500	45,169	0	0.990
	Abnormal	0.508	24,097	7.213	0.119	0.497	5,187	9.164	0.010
	Normal	0.502	55,492			0.501	39,982		
17	All	0.502	76,327	9.821	0.007	0.500	45,169	0	0.889
	Abnormal	0.517	18,489	0	0.993	0.480	2,755	4.154	0.111
	Normal	0.498	57,838			0.502	42,414		
18	All	0.503	88,607	0	0.796	0.500	45,169	0	0.927
	Abnormal	0.510	23,587	2.717	0.204	0.481	3,168	5.080	0.073
	Normal	0.500	65,020			0.502	42,001		

Table 3. Cont.

		FISH				aCGH			
Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight	CSR	*N*	ΔAIC	Akaike weight
19	All	—	—	—	—	0.500	45,169	0	0.995
	Abnormal	—	—	—	—	0.492	4,499	10.459	0.005
	Normal	—	—	—	—	0.501	40,670		
20	All	0.502	17,866	0	0.969	0.500	45,169	0	0.975
	Abnormal	0.497	4,896	6.910	0.031	0.486	3,213	7.332	0.025
	Normal	0.504	12,970			0.501	41,956		
21	All	0.503	89,669	0	0.973	0.500	45,169	0	0.987
	Abnormal	0.510	25,434	7.151	0.027	0.496	4,362	8.624	0.013
	Normal	0.500	64,235			0.501	40,807		
22	All	0.504	80,548	0	0.992	0.500	45,169	1.441	0.327
	Abnormal	0.503	25,218	9.567	0.008	0.480	5,098	0	0.673
	Normal	0.504	55,330			0.503	40,071		

studies because the diagnosis of sex from morphology likely overestimates the CSR, especially in early pregnancy (47, 48), due to the difficulty of distinguishing between female and male genitalia of early fetuses. We regard the chromatin estimate and especially the karyotype estimate as much more accurate; for these, CSR increases with CA (Fig. 1), which is consistent with greater net mortality for female fetuses during the first two trimesters. The male bias of the chromatin trend compared with the karyotype trend is consistent with the claim that the former method overestimates the CSR because female cells with poor staining of the Barr body are falsely classified as male.

Analysis of CVS Data. We assessed whether the abnormal and the normal CSR differed by using a fixed-effect analysis because there was only one sample per mother (Table 7). The stratified and unstratified models have similar support (ER ~ 1.61). The CSR is more male-biased (0.514) compared with the CSR among embryos (0.502; Table 1). Approximately 9% of fetuses were abnormal during this period compared with ~61% among embryos (Table 1).

We also used a fixed-effect regression analysis to assess the relationship between the CSR and CA (Table 8). The model without CA as predictor has greater support (ER ~ 8.52); this model indicates that the CSR increases between 6 and 12 wk (Fig. 2).

Analysis of Amniocentesis Data. We assessed whether the abnormal and the normal CSR differed by using a fixed-effect analysis because there was only one sample per mother (Table 9). The stratified model has much greater support (ER > 1,000), which suggests that the abnormal and normal CSRs are distinct. The CSR is less male-biased (0.506) compared with the CSR among CVS fetuses (0.514). Approximately 3.5% of embryos are abnormal; the abnormal CSR estimate is male-biased.

We also used a fixed-effect regression analysis to assess the relationship between the CSR and CA (Table 10). The model with CA as predictor has much greater support (ER > 1,000); this model indicates that the CSR increases between 10 and 20 wk (Fig. 3).

There could be an overrepresentation of females among the fetuses undergoing amniocentesis, especially among early procedures, because there is a higher false-positive rate among females in tests for chromosome 21 aneuploidy based on maternal serum levels of α-fetoprotein (AFP) and free β-human CG (β-hCG) (49). Such a bias could generate an increasing relationship between the CSR and CA. The CSR of screened pregnancies in our sample is less male-biased than for unscreened pregnancies. However, the CSR increases between 10 and 20 wk for screened pregnancies and for unscreened pregnancies. We conclude that maternal screening does not distort our qualitative understanding of the CSR.

Analysis of Fetal-Death and Live-Birth Data. Karyotypic information for fetuses and babies is very limited. We did not distinguish between the abnormal CSR and the normal CSR. The CSR declines markedly after 35 wk CA (Fig. 4) due to the tendency of males to be born earlier. The birth sex ratio can be viewed as an admixture of an earlier male-biased wave followed by a female-biased wave. This shift is no fluke of sampling; there were 17,309,547 births and fetal deaths during weeks 35–37 and 14,010,729 thereafter.

The trend of the CSR estimates when CA is based on the clinical estimate is virtually identical to that shown in Fig. 4 up to 33 wk. The CSR then declines until week 38, but it never becomes female-biased. The estimates for later CAs are very variable, perhaps because there are many fewer pregnancies with late clinical estimates, especially those greater than or equal to 41 wk (clinical: $n = 27{,}567$; LMP: $n = 1{,}309{,}690$). We do not view the greater stability of late LMP-based CSR estimates as a reason to prefer this dating method; we urge further research to resolve the controversy over dating methods (50–52).

Discussion

Analysis of ART Embryos. Sex-biased mortality may have occurred before assay, although this is unlikely. Such mortality could be caused by disrupted expression of maternally inherited mRNA or of RNA synthesized by the embryo. The ART embryos had at least eight cells when assayed. Some gene expression starts at the one-cell stage, and some X- or Y-linked loci are expressed before the eight-cell stage (53–58); embryonic genome activation is reviewed by refs. 59 and 60. It is implausible that any such differential mortality just happens to produce an assemblage of embryos whose CSR is statistically coincident with 0.5, a value expected given unbiased segregation of sex chromosomes during spermatogenesis and unbiased fertilization. An exact a posteriori

Table 4. Mixed-effect analyses of the association between MA and the CSR, as estimated from ART embryos analyzed by FISH

Model	Fitted model	ΔAIC	Akaike weight
I	Logit(CSR) = 0.012	0	0.971
I + MA	Logit(CSR) = -0.075 + 0.002MA	7.043	0.029

I denotes intercept. $n = 92{,}037$.

Table 5. Mixed-effect analyses of the influence of trimester on the CSR estimated from induced-abortion data

Sample of fetuses	CSR	*N*	ΔAIC	Akaike weight
All with known trimester	0.524	4,999	4.737	0.086
First trimester	0.511	3,392	0	0.914
Second trimester	0.559	1,607		

Table 6. Mixed-effect analyses of the influence of CA on the CSR estimated from induced-abortion data

Model	Fitted model	*N*	ΔAIC	Akaike weight
I + CA	$Logit(CSR) = 0.063 + 0.006CA_{early}$	14,839	7.322	0.025
I + CA method-specific	$Logit(CSR)_C = 0.086 + 0.005CA_{early}$	8,373	0	0.975
	$Logit(CSR)_K = -0.157 + 0.013CA_{early}$	4,872		
	$Logit(CSR)_M = 0.852–0.044CA_{early}$	1,594		

I denotes intercept, $_C$ denotes chromatin, $_K$ denotes karyotype, and $_M$ denotes morphology. $_{early}$ denotes analyses based on early conception ages (see text).

power calculation provides additional insight. Assume that the (false) null hypothesis is that the CSR is 0.5 and that the (true) alternative hypothesis is that the CSR is, say, 0.505. For n = 139,704, when $\alpha = 0.05$, there is an ~59% statistical power to reject the false hypothesis that the CSR is 0.5. If the true CSR is 0.510, there is an ~98% power to reject the false hypothesis.

There are nine reasons why ART embryos provide a meaningful estimate of the CSR and why our unbiased estimate of the PSR is plausible; we list them in rough order of their importance. Details are provided in *SI Text*.

i) The birth sex ratio of babies conceived via ART matches the birth sex ratio of babies conceived naturally.
ii) The birth sex ratio for ART with in vivo conception and the birth sex ratio for ART with in vitro conception appear to be identical.
iii) Our estimate of the PSR matches the value expected given unbiased segregation of sex chromosomes during spermatogenesis and unbiased fertilization.
iv) Analyses of data from other species do not provide conclusive evidence that the mammalian PSR is male-biased.
v) The method of in vitro conception does not appear to influence the ART estimate of the CSR.
vi) A high proportion of early naturally conceived embryos may be abnormal (as in our ART sample).
vii) Typical methods for collection and preparation of gametes appear to have little or no influence on the ART birth sex ratio.
viii) The average age difference between women who use ART and women who conceive naturally does not imply that ART embryos are unsuitable as a basis for an estimate of the PSR.
ix) Ionic strength, pH, and temperature during fertilization and early development vary across ART protocols but are not grossly different from in vivo conditions as far as they are known.

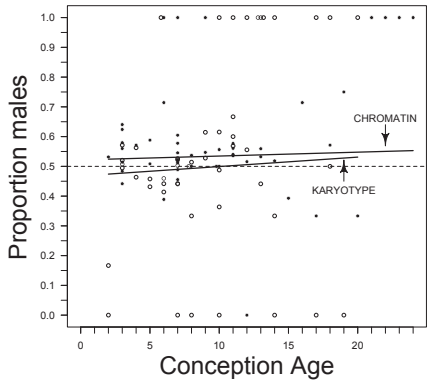

Fig. 1. The relationship between conception age and cohort sex ratio estimated from induced-abortion data. Observed sex ratios and estimated regression for chromatin (●) and for karyotype (○) data (Table 6). A dashed line denotes a sex ratio of 0.5.

Analysis of XO Embryos. ART embryos with one X chromosome and no Y chromosome (XO) were not included in our CSR estimate because their sex is ambiguous; the many fewer YO embryos were included. Each XO embryo may never have had a maternal and a paternal sex chromosome or it may have lost one. The latter kind of embryo should contribute to a CSR estimate. We calculated their potential influence on the CSR estimate derived from the FISH analyses as follows. The percentage of XO embryos having a maternal X chromosome may be similar to the live-born frequency, which is at least 75% (61) [there is only one study of XO embryos known to us; all had a maternal X chromosome, n = 10]. If true and XO embryos had equal probabilities of resulting from X- and Y-bearing sperm, one expects that 62.5% of XO embryos were female (XX) and 37.5% were male (XY). There were 11,372 XO samples in our ART sample. The argument above implies that there are more "hidden" females (at most 7,107.5) than hidden males (at most 4,264.5). Accordingly, any correction for the missing embryos will leave unchanged or reduce the CSR estimate. For example, if h is the proportion of hidden zygotes in the XO sample, when h = 0 (no hidden zygotes), the CSR estimate is 0.502 [=70,171/(70,171 + 69,533)], which is the CSR estimate in Table 1. When h = 0.5, the CSR estimate is 0.497 {=[70,171 + 0.5(4,264.5)]/[70,171 + 69,533 + 0.5(11,372)]}. When h = 1.0, the CSR estimate is 0.493 = (70,171 + 4,264.5)/(70,171 + 69,533 + 11,372). We believe that the value of h for our sample is closer to 1.0 than to 0.0; most XO embryos had two copies of at least several chromosomes. No matter what the value of h, these estimates demonstrate that inclusion of hidden zygotes from among the XO sample does not generate a male bias in the CSR estimate.

This argument implies that our abnormal CSR estimate in Table 1 (0.508, n = 84,881) is based on a sample from which abnormal females were 66% (=62.5/37.5) more likely than abnormal males to be excluded. When h = 0, the abnormal CSR estimate is 0.508 [=43,144/(43,144 + 41,737)], which is the estimate in Table 1. When h = 0.5, the abnormal estimate is 0.500 {=[43,144 + 0.5(0.375)(11,372)]/[43,144 + 0.5(0.375)(11,372) + 41,737 + 0.5(0.625)(11,372)]}. When h = 1.0, the estimate is 0.493 {=[43,144 + (0.375)(11,372)]/[43,144 + (0.375)(11,372) + 41,737 + (0.625)(11,372)]}. The normal CSR estimate remains female-biased (0.493 in Table 1). None of the corrections of the CSR or of the abnormal CSR suggest that there is a substantial male bias of the PSR or of the CSR during early pregnancy.

Possible Causes of the Influence of Specific Chromosomes. The association between CSR estimates and the state of the sex chromosomes and of chromosome 15 (Table 3, FISH) may be caused by entanglement of the bivalents of the Y chromosome

Table 7. Fixed-effect analyses of the influence of karyotypic state on the CSR estimated from CVS data

Fetuses	CSR	*N*	ΔAIC	Akaike weight
All	0.514	61,769	0	0.617
Abnormal	0.521	5,481	0.956	0.383
Normal	0.513	56,288		

Table 8. Fixed-effect analyses of the influence of CA on the CSR estimated from CVS data

Model	Fitted model	ΔAIC	Akaike weight
I	Logit(CSR) = 0.053	4.294	0.105
I + CA	Logit(CSR) = -0.218 + 0.023CA	0	0.895

I denotes intercept. n = 60,081.

Table 9. Fixed-effect analyses of the influence of karyotypic state on the CSR estimated from amniocentesis data

Fetuses	CSR	N	ΔAIC	Akaike weight
All	0.506	839,590	44.814	<0.001
Abnormal	0.523	36,833	0	>0.999
Normal	0.505	802,757		

and those of chromosome 15 at the pachytene stage of meiosis I. There is sequence homology between repetitive DNA in the heterochromatin of chromosome 15 and the heterochromatin of the q arm of the Y chromosome (62, 63). Such homology likely generates a physical association between the sex vesicle or "XY body" (64, 65) and the short arm of chromosome 15; physical association likely also occurs during metaphase (66). Sequence homology between repetitive DNA in chromosome 15 (and the other acrocentric chromosomes: 13, 14, 21, and 22) and in the X chromosome may also help generate a physical association (67); this may cause the excess of translocations involving the X chromosome and chromosomes 15, 21, and 22 (68). Entanglement may underlie the susceptibility of chromosome 15 to karyotypic abnormalities (69).

Karyotypic abnormalities generated in spermatogenesis, although rarer than those generated during oogenesis, may have a special influence on early development (70). Chromosomes 7 (Table 3, aCGH) and 17 (Table 3, FISH) may also exhibit this influence, although we lack possible causal explanations at the molecular level. Abnormality involving chromosome 7 (uniparental disomy that may disrupt imprinting; polysomy) is known or suspected to be associated with male-biased pathology after birth (71, 72), but the association of this chromosome with sex-specific prenatal morbidity and mortality appears not to have been investigated. An association between the Y chromosome and disomy for chromosome 21 has been described in sperm by ref. 73, although its cause is unknown (74, 75). This association is consistent with the decrease in the male bias of the abnormal CSR estimate for chromosome 21 (Table 3, FISH: 0.510 vs. aCGH: 0.496), although there is equivocal support for either stratified model. The apparent lack of influence of the state of chromosomes 13 and 18 on the CSR suggests that sex ratio biases among newborns aneuploid for these chromosomes are due to mortality during later development, as suggested by refs. 76 and 77.

Our assessments of the association between specific chromosomes and the abnormal and normal CSR estimates are based on Akaike weights (Table 3). For the FISH data, these assessments are identical to those based on adjusted P values derived from the change in deviance between nonstratified and stratified models [adjustments were based on a Bonferroni correction that controls in the weak sense the familywise type 1 error rate at 0.05 or on a correction that controls the false-discovery rate (78, 79) at 0.05]. For the aCGH data, Akaike weights, a Bonferroni correction, and a correction of the false discovery rate underwrite identical conclusions for all chromosomes except chromosome 7 (ΔAIC = 4.400, $P_{\text{Bonferroni}} = 0.305$, $P_{\text{False-discovery-rate}} = 0.152$).

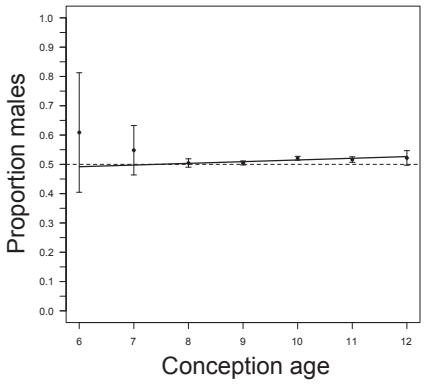

Fig. 2. The relationship between conception age and cohort sex ratio estimated from CVS data. Observed cohort sex ratio (with 95% confidence limits) and the estimated regression (Table 8). Fractional ages are rounded to the nearest integer. A dashed line denotes a sex ratio of 0.5.

Analysis of Induced-Abortion Data. Our analysis suggests that female-biased mortality causes the CSR to increase between 2 and 20 wk CA. This increase is consistent with the inference from the ART analysis that the early CSR could be female-biased. Induced-abortion studies reporting female-biased first-trimester CSR estimates appear to be carefully done (17, 80–85). In addition, refs. 48 and 86–88 described female-biased CSRs for first trimester spontaneous abortions, but see ref. 89.

Analysis of CVS Data. Our analysis suggests that the CSR is female-biased early in pregnancy and that female-biased mortality causes it to increase between 6 and 12 wk CA.

Analysis of Amniocentesis Data. Our analysis suggests that the CSR increases between 10 and 20 wk due to female-biased mortality and that it surpasses 0.5 at ~15 wk CA.

Analysis of Fetal-Death and Live-Birth Data. Male-biased mortality during the second half of the second trimester and during the third trimester has little influence on the CSR (Fig. 4); the small size of this influence appears to be underappreciated.

The biphasic nature of the sex ratio of births (Fig. 4) has not been investigated thoroughly (90–92), although it has important implications for how to define a "premature" birth. One proximate cause of the sex ratio change may be that males typically attain a critical fetal weight earlier than do females (the average weight of newborn males is ~100 g greater than females in the US data). Birth initiation is discussed in refs. 93 and 94.

James claimed that there is "a [positive] association of male births with long gestations" (95, p. 264) and that there is an "excess of males among post-term births" (92). A postterm birth is defined as one having a CA of 38 wk (40 wk LMP) or greater. For the US data, the CSR estimate for all post–38-wk births is 0.493 (95% CI: 0.493–0.493, n = 6,573,562), which is lower than the estimate for week 38 (0.497, 95% CI: 0.497–0.497, n = 7,437,167), suggesting an opposite trend, if any, to the one posited by James.

Overview. Our analysis suggests that the PSR is unbiased. Analysis of the ART data suggests that the CSR could become female-biased within a week or two of conception because more male embryos are abnormal (assuming that the death rate of abnormal male embryos during this period is at least equal to that of abnormal female embryos). The CSR then increases early in

Table 10. Fixed-effect analyses of the influence of CA on the CSR estimated from amniocentesis data

Model	Fitted model	ΔAIC	Akaike weight
I	Logit(CSR) = 0.022	168.522	<0.001
I + CA	Logit(CSR) = -0.241 + 0.017CA	0	>0.999

I denotes intercept. n = 809,274.

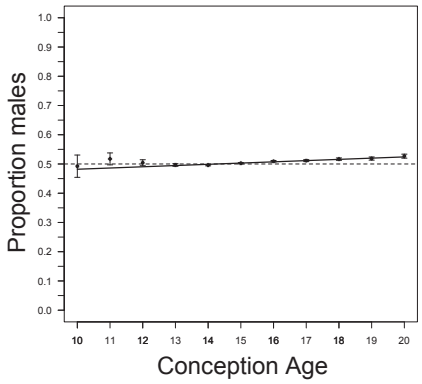

Fig. 3. The relationship between conception age and cohort sex ratio estimated from amniocentesis data. Observed cohort sex ratio (with 95% confidence limits) and the estimated regression (Table 10). Fractional conception ages are rounded to the nearest integer. A dashed line denotes a sex ratio of 0.5.

pregnancy (due to higher female mortality) and decreases later in pregnancy (due to higher male mortality). Three independent datasets (induced abortions, CVS, and amniocentesis) suggest that the CSR increases until the latter half of the second trimester. If the PSR is 0.5, total female mortality must be greater than total male mortality during pregnancy because the sex ratio of all births is male-biased.

Female-biased mortality during the second trimester is likely not caused by gross karyotypic abnormalities such as monosomy and trisomy, because these probably cause earlier death. A female bias has been reported among apparently karyotypically normal spontaneous abortions during the first two trimesters (86–88). The apparent increase in female mortality occurs despite gene expression by two X chromosomes (although most loci on one or the other X chromosome are not expressed in a given cell). The expression of deleterious mutations is thought to be masked when the two X chromosomes have equal inactivation probabilities (96). Sex differences in gene expression are known later in pregnancy and later in life (97–99), but we lack information on how sex differences in gene expression earlier in pregnancy might contribute to female-biased mortality. One possible mechanism is that a paternal X chromosome retards development in such a way that female mortality rate increases; this has been confirmed in the mouse (100). Another possible mechanism is skewed X-inactivation (usually defined as >75% of cells sampled having, say, the paternal X chromosome inactivated), which can unmask recessive deleterious alleles (101, 102); it can also mask them (103). Skewed inactivation is associated with female-biased pathology later in life (104–106) and also with an elevated risk of spontaneous abortion (107, 108), although the sex ratio of the abortions appears to be unknown.

There are ambiguities in regard to our estimate of the trajectory of the CSR from conception to birth (Fig. 5). One is the discrepancy among the quantitative estimates of the CSR between 10 and 20 wk. A likely cause of the female bias of the amniocentesis estimates compared with the induced-abortion estimates is the presence of more than 200,000 fetuses in our sample that have undergone amniocentesis due to elevated AFP and total hCG levels (see above). When such fetuses are excluded, CSR estimates are higher than those in Fig. 3 and are consistent with those from induced abortions. For example, among fetuses whose rounded conception age is 20 wk, the CSR for those with elevated AFP and total hCG levels is 0.492 (n = 8,598) and 0.552 (n = 11,873) for the others. The latter estimate is close to the CSR at 20 wk inferred from the induced-abortion data.

We now address James' causally explicit claim (109, 110) that more males than females are conceived due to the interaction between the timing of fertilization and fluctuations of estrogen, testosterone, gonadotrophins, and progesterone during the menstrual cycle. The key assumption of this hypothesis is that the male-biased birth sex ratio is the result of a male-biased PSR. Such backward extrapolation is potentially misleading, and in this instance, the analysis of the induced-abortion data indicates that the CSR is female-biased during the first trimester of pregnancy and only later becomes male-biased. We do not deny the reality of the hormonal fluctuations and the nonuniformity of fertilization times, although whether the birth sex ratio depends on hormonal fluctuations is controversial (111–115). Even if there is such a dependency, the birth sex ratio does not have any necessary implication for the PSR; perhaps, for example, the timing of conception has a differential effect on the fate of male and female embryos (116). We conclude that James' claim is incorrect, given our results that the PSR is unbiased, that the CSR may be female-biased during the first trimester, that the CSR increases during the first trimester, and that the predicted male bias among postterm births is absent.

Our results are also inconsistent with the hypothesis that the male-biased birth sex ratio arises from male-biased implantation of blastocysts after unbiased conception (117). The CSR early in the first trimester (after implantation) could be female-biased and the CSR increases during the first two trimesters. To this extent, male-biased implantation cannot by itself explain the male-biased birth sex ratio. In addition, the normal CSR estimate for the aCGH embryos is not male-biased (Table 3, Any = 0.498). Most of these embryos had undergone blastocyst formation, which may indicate competency for implantation.

We now consider the implications of our results for understanding of the evolution of the human sex ratio.

Extending the argument of Düsing (118), Fisher (8) claimed that the sex ratio had evolved via a process of natural selection and that the equilibrium outcome of this process is equal investment in the two sexes at "the end of the period of parental expenditure." Fisher implied that there is a monotonic trajectory of the CSR towards this equilibrium; this is contradicted by our results (see Fig. 5 and *SI Text*).

We address two specific claims as to the sex ratio associated with this equal investment equilibrium (see *SI Text*). First, many scientists believe that 0.5 is the equilibrium sex ratio, although Fisher did not make this specific claim. We show using US data that the sex ratio for the 1900 cohort at age 40 is consistent with 0.5. However, the evolutionary implications of this result are ambiguous given the lack of real data on the sex specificity and timing of investment. This ambiguity is an important cautionary lesson, which is underscored by our result that female mortality during pregnancy may be greater than male mortality. All other things being equal, this greater female mortality implies that the sex ratio at investment equilibrium should be male-biased.

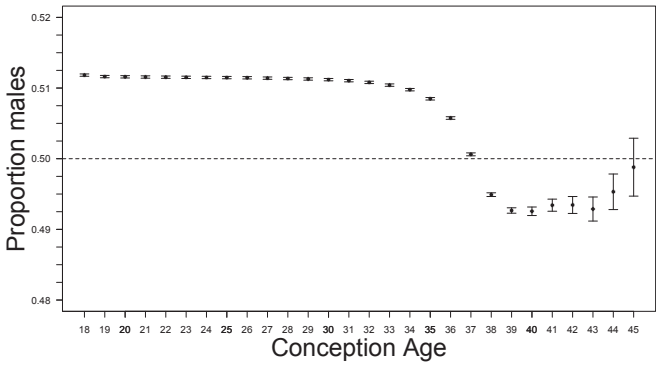

Fig. 4. The relationship between conception age and cohort sex ratio estimated from US fetal deaths and live births for 1995–2004 (combined). Observed cohort sex ratio (with 95% confidence limits). Conception age is based on the date of the last menstrual period; 18 denotes ≤18 wk. A dashed line denotes a sex ratio of 0.5.

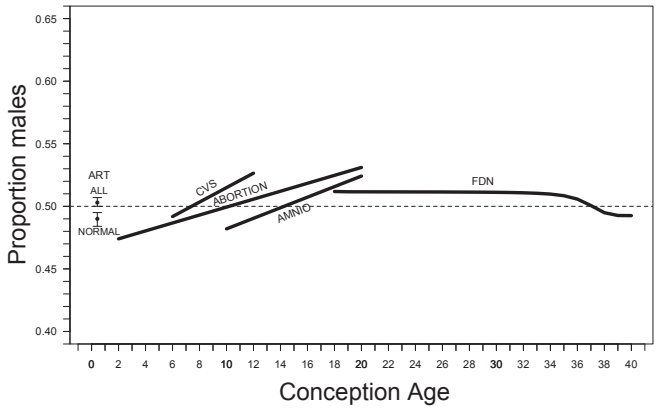

Fig. 5. The trajectory of the cohort sex ratio from conception to birth. ALL and NORMAL denote the total and normal sex ratio estimates based on ART embryos (Table 1), respectively, CVS denotes the estimated sex ratio trend based on CVS data (Table 8), ABORTION denotes the estimated trend based on induced abortions sexed via karyotype (Table 6), AMNIO denotes the estimated trend based on amniocentesis data (Table 10), and FDN denotes the trend of cohort sex ratio based on US fetal deaths and live births. A dashed line denotes a sex ratio of 0.5.

Second, we show that Charlesworth's (119) prediction that the equilibrium sex ratio is female-biased (p. 356) by "the end of the first year of postnatal life" for populations with little or no post-birth investment is not consistent with the data from the 1900 cohort or with data from hunter-gatherer, horticultural, and pastoral societies (120).

Finally, we suggest (see *SI Text*) that it is not self-evident that the sex ratio of a human cohort attains any fixed value (apart from sampling error) before only one sex remains. Static idealization of a trait can be misleading if dynamic expression is a central component of a trait's evolutionary response to natural selection (121, 122). Determining the validity of this static idealization that the ultimate target of natural selection is a single sex ratio (as opposed to the target being, say, an age-specific sequence of sex ratios) will require data on the sex specificity and timing of parental investment, statistical assessment of the age-specific sex ratios to determine whether they are reasonably regarded as age invariant, and a comparison of the predictive accuracy of relevant static and dynamic adaptive models.

ACKNOWLEDGMENTS. We thank two anonymous reviewers, Paul Bain, Martin Bobrow, Ben Bolander, Ben Bolker, Fred Burchsted, Dan Chasman, Ariane Cherbuliez, Jean Gladstone, Andrew Guarnaccia, Elena Labarta, Jeffrey Lebowski, Ron Lee, Richard Lewontin, Marian Macdorman, Renée Martin, Brian McLoone, Erik Moore, Sterling Puck, Lia Ribustello, Lorena Rodrigo, Barbara Rolfe, Luke Rogers, the Ronin Institute, Seymour Sacks, Mary Sears, Gill Shaddick, Korbinian Strimmer, Henri Termeer, Syzmon Tolak, Giles Tomkin, and Nathan Treff for assistance. This project was partially supported by Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant R03HD055685, and the National Academies Keck Futures Initiative.

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Supporting Information

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SI Text

FISH Probes Used to Karyotype ART Embryos. The FISH probes and their target locus and region (both in parentheses) were X chromosome: CEP X (DXZ1, p11.1-q11.1), Y chromosome: CEP Y Alpha Satellite at Genzyme Genetics (DYZ3, p11.1-q11.1), and CEP Y Satellite III at Reprogenetics (DYZ1, q12), chromosome 8: CEP 8 (D8Z2, p11.1-q11.1), chromosome 9: CEP 9 Alpha Satellite at Genzyme Genetics (unknown, p11.1-q11), chromosome 13: LSI 13 (RB1, q14.1-q14.3), chromosome 14 at Reprogenetics: TelVysion 14q (STS-X58399/SHGC-36156/STS/AA034492/telomeric IGHV segments, q32.3), chromosome 15: CEP 15 Alpha Satellite (D15Z4, p11.1-q11.1), chromosome 16: CEP 16 Satellite II (D16Z3, q11.2), chromosome 17: CEP 17 at Reprogenetics (D17Z1, p11.1- q11.1), chromosome 18: CEP 18 (D18Z1, p11.1-q11), chromosome 20 at Reprogenetics: TelVysion 20p (D20S1157, p13), chromosome 21: LSI 21 (D21S259/D21S341/D21S342, q22.13-q22.2), and chromosome 22: LSI 22q (BCR, q11.2). Details of sample preparation and protocols are available on request (see refs. 1 and 2 for protocols used at Reprogenetics). All probes were obtained from Abbott Molecular (www.abbottmolecular.com).

Summary of Induced Abortion Studies. The 41 studies of the sex ratio of induced abortions are shown in Table S1.

Procedures Used to Process CVS and Amniocentesis Samples. Cells were cultured following refs. 3–5. Cell suspensions were placed on coverslips in Petri dishes containing growth media. After 5–10 d, a mitotic inhibitor (colcemid) was added. Cells were harvested by removing the media and mitotic inhibitor and adding a hypotonic solution, followed by changes of fixative (3:1 methanol to acetic acid). The cells were dried, thereby breaking the nuclei of dividing cells and spreading the chromosomes. After treatment with trypsin, chromosomal bands were visualized with Wright-Giemsa stain. Images of at least four metaphase cells per sample were recorded, and karyotypes were recorded for two or three cells.

Week-Specific Estimates of the CSR Based on Fetal-Death and Live-Birth Data for the US 1995–2004. Data for weeks postconception (CA) based on LMP are shown in Table S2.

Mixed-Effect Analyses of the Association Between the State of Individual Chromosomes in ART Embryos and the Cohort Sex Ratio. Analyses of the combined FISH and aCGH data are shown in Table S3.

Mixed-Effect Analyses of the Association Between the Overall State of the Embryo (Any) or the State of Individual Chromosomes and the Cohort Sex Ratio. Analyses of the aCGH data for blastomere samples and blastocyst samples are shown in Table S4.

Mixed-Effect Analyses of the Association Between the Overall State of the Embryo (Any) or the State of Individual Chromosomes and the Cohort Sex Ratio. Analyses of blastomere samples (FISH only) and blastocyst samples (aCGH) are shown in Table S5.

Nine Reasons Why ART Embryos Provide a Meaningful CSR Estimate. ***The birth sex ratio of babies conceived via ART matches the birth sex ratio of babies conceived naturally.*** The birth sex ratio arising from our sample of ART embryos is unknown. We analyzed data from the Australian Institute of Health and Welfare (www.npesu.unsw.edu.au/surveillance-reports); this is the largest comparison of ART and natural sex ratios to date. As shown in Table S6, the sex ratio of ART births (0.515, 95% CI: 0.512–0.517, n = 136,647) and the sex ratio of natural births (0.514, 95% CI: 0.514–0.514, n = 5,500,467) are statistically identical. These estimates match previous results. Ref. 6 (table 3) reported an ART birth sex ratio for Denmark from 1995 to 2000 of 0.521 (95% CI: 0.511–0.531, n = 8,894) and a sex ratio for all births from 1995 to 2004 of 0.513 (95% CI: 0.512–0.515, n = 663,276). Other smaller studies reporting this overlap include refs. 7–10. However, ref. 11 (p. 1582) reported an ART sex ratio of 0.498 (95% CI: 0.490–0.506, n = 15,164) and a sex ratio for 2005 US births of 0.512 (95% CI: 0.511–0.512, n = 4,138,349).

Our overall conclusion is that ART generates a cohort of fetuses whose fates during pregnancy match those of naturally conceived fetuses.

The birth sex ratio for ART with in vivo conception and the birth sex ratio for ART with in vitro conception appear to be identical. We assessed the influence of in vivo vs. in vitro conception by comparing standard ART and gametic intrafallopian transfer (GIFT) birth sex ratios. This comparison holds constant the influence of in vitro treatment of eggs and sperm; standard ART involves a variety of artificial conception methods and GIFT involves natural conception. We analyzed data collected by the Australian Institute of Health and Welfare. As shown in Table S7, the sex ratio for GIFT is 0.521 (95% CI: 0.511–0.531, n = 9,312) compared with the estimate for ART (0.515, 95% CI: 0.512–0.517; Table S6); almost all of the ART births involved IVF and ICSI and not GIFT. We conclude that there is no influence of in vitro conception per se on the birth sex ratio.

Our estimate of the PSR matches the value expected given unbiased segregation of sex chromosomes during spermatogenesis and unbiased fertilization. We further note that this match occurs despite geographic and temporal heterogeneity of samples (embryos came from ART clinics across the United States and other countries between 1995 and 2009). There is no evidence that spermatogenesis results in a ratio of X- and Y-bearing sperm similar to the sex ratio bias among births. Instead, studies suggest that spermatogenesis results in an unbiased ratio of X- and Y-bearing sperm (12–15) or perhaps a slight bias (toward X chromosome-bearing sperm) (16–18). In addition, segregation of other human chromosomes appears to be unbiased.

Analyses of data from other species do not provide conclusive evidence that the mammalian PSR is male-biased. There are nonmolecular estimates (derived from sex chromatin or karyotyping) and molecular estimates. The nonmolecular estimates should be interpreted cautiously for four reasons. First, scoring sex chromatin likely overestimates the number of males (19). Second, some estimates are based on fetal morphology, which can be unreliable, especially for early fetuses. Third, some estimates are based on an amalgamation of embryos and fetuses. Fourth, some studies based their estimate only on the sex ratio at birth. The molecular estimates involve protein-based and DNA-based techniques (20, 21). Estimates are shown in Table S8.

We analyzed these data (without phylogenetic correction) with a mixed-effect analysis in which studies within species were treated as random effects and species were treated as factors. We analyzed the nonmolecular data and the molecular data separately; in both cases, there is substantially more support for the model with an overall sex ratio compared with the species-specific model. The overall nonmolecular estimate is 0.531 (95% CI: 0.516–0.547), and the overall molecular estimate is 0.498 (95% CI: 0.485–0.512). The latter, more reliable, estimate does not provide compelling evidence that the PSR is male-biased in mammals.

We note that there is also no indication that the sex ratio at birth in mammals is usually male-biased (22, p. 400).

The method of in vitro conception does not appear to influence the ART estimate of the CSR. The method of conception is known for a subset of embryos in our FISH sample (n = 8,214). These embryos were conceived via standard ART (IVF) or via intracytoplasmic sperm injection (ICSI). We assigned random effects to women and treated method of conception as a factor (this sample contained only a single procedure for each woman). Support for the two models is comparable; the overall CSR is 0.508 (95% CI: 0.496–0.519, n = 8,214); this is similar to the estimate for the entire sample (0.502) in Table 1. The IVF estimate is 0.518 (95% CI: 0.502–0.533, n = 4,361), and the ICSI estimate is 0.496 (95% CI: 0.480–0.513, n = 3,853). Neither conception method is the same as natural conception, but we caution against simple conclusions as to which one is more like natural conception, especially given the lack of evidence for a difference in the associated sex ratios.

A high proportion of early naturally conceived embryos may be abnormal (as in our ART sample). A high proportion of abnormal ART embryos has been previously reported (23, 24). Very few naturally conceived embryos less than 1 wk old have been studied, but some authors reported abnormalities (25–38); to our knowledge, none of these embryos has been karyotyped.

There are three kinds of circumstantial evidence that many naturally conceived embryos are karyotypically abnormal. First, possibly up to 70–80% of conceptions fail (even among young mothers). Perhaps 50% fail subclinically within the first few weeks (39–61). Much mortality may be caused by an abnormal karyotype (57, 62); many spontaneous abortuses have karyotypic abnormalities (63–73). Second, oogenesis is error prone (74–77). Spermatogenesis appears to be less error prone; a few percent of sperm are abnormal (15). Karyotypically abnormal gametes can form zygotes (78–82). Third, mitotic errors occur frequently in cleavage-stage embryos and in blastocysts (56, 83, 84). Limited evidence suggests that the frequencies of karyotypic abnormalities in embryos conceived in vitro and in vivo differ in some species (85, 86) but not all (87).

Typical methods for collection and preparation of gametes (88, 89) appear to have little or no influence on the birth sex ratio. For example, it is likely that many embryos in our sample were derived from oocytes collected after ovarian stimulation via gonadotropin or clomiphene citrate (90). Limited data indicate that the birth sex ratio after such stimulation (but with natural conception) does not differ from the sex ratio without stimulation (91). The typical techniques used to capacitate sperm have little influence on the sex ratio of ART births (92). In addition, limited data indicate that embryos derived from unstimulated oocytes and those derived from stimulated oocytes have similar frequencies of abnormality (93).

The average age difference between women who use ART and women who conceive naturally does not imply that ART embryos are unsuitable as a basis for an estimate of the PSR. Women who use ART are not a random sample of pregnant women. For example, the average mother's age in our sample is 36.6 y, which is older than the average mother's age in the United States. However, young women who use ART, but not for fertility problems, produce a high percentage of karyotypically abnormal embryos (94, 95), which suggests that age and fertility problems do not cause this high percentage (96, 97). It is believed that most such embryos arise from abnormal oocytes and that the rate of meiotic aneuploidy in oocytes increases with age (98). However, such an increase has not always been observed (99). In addition, aneuploidy increases linearly with age for some chromosomes (100, 101), whereas for others, it increases only after age 40 y (102).

Ionic strength, pH, and temperature during fertilization and early development vary across ART protocols but are not grossly different from in vivo conditions as far as they are known (103–105). Much progress has been made at characterizing in vivo conditions (106–110). We know of no evidence that known differences between in vitro and in vivo conditions affect the in vivo sex ratio (111) or that in vitro conditions affect the birth sex ratio. However, we acknowledge that even small differences between in vitro and in vivo conditions might cause a difference in their associated sex ratios.

The Implications of Our Results for Understanding of the Evolution of the Human Sex Ratio. Extending the argument of Düsing (112), Fisher (113) claimed that the evolutionary equilibrium resulting from the long-term process of natural selection on the sex ratio was equal investment in the two sexes at "the end of the period of parental expenditure." The evolution of this equilibrium is driven by a Darwinian dynamic in which individuals or couples whose heritable investment in the two sexes is closer to equal gain higher representation in the population over the long-term. All other things being equal, this process of selection among individuals or couples stops when the evolutionary equilibrium of equal investment is attained, i.e., the population as a whole invests equal amounts into the two sexes of offspring (114, 115). Specific assumptions are needed in order to generate the prediction that an individual or a couple produce equal investment when the population is at the equal investment equilibrium (116).

Fisher claimed that the human sex ratio has evolved to an equal investment equilibrium at the end of parental expenditure via the Darwinian process described above. He did not state at what age of offspring the end occurs. However, he did describe the trajectory of the sex ratio of a cohort from conception to the equal investment equilibrium. He stated that more males are conceived than females and implied that the equilibrium is approached monotonically due to higher mortality of males between conception and the end of parental expenditure (p. 159). Fisher did not specifically predict that the sex ratio is 0.5 when parental expenditure ends (this prediction depends on assumptions about energy investment and mortality schedules that may not be true for humans); nonetheless, many scientists believe that this sex ratio is the outcome predicted by Fisher. Our results suggest that the CSR starts at 0.5, becomes female-biased, reattains 0.5, becomes male-biased, and decreases past 0.5. Whatever equilibrium one might specify, this trajectory indicates that the CSR does not exhibit a monotonic trajectory like the one implied by Fisher.

We can still heuristically assess whether the equal investment equilibrium is attained in a human population. We stress that data on the sex specificity and timing of investment are required if any claims are to go beyond crude speculation. Equal investment is predicted for age-structured populations (117), given random mating of individuals of different ages and little or no influence of parental age on the sex ratio produced. We assume that the net energetic cost of a son and of a daughter are equal at the end of parental investment; this implies that the sex ratio will be 0.5 at that age. We also assume that data from a single cohort are sufficient to test this prediction.

Age-specific estimates of the sex ratio can be obtained using the estimated numbers of males and females resident in the US who were born in 1900 (Table S9); their sex ratio trajectory is essentially complete. (Data for ages 0–79 y are available at www.census.gov/popest/data/national/asrh/pre-1980/PE-11.html. Data for ages 80–89 y are available at www.census.gov/popest/data/national/asrh/1980s/80s_nat_detail.html, and data for ages 90–99 y are available at www.census.gov/popest/data/intercensal/national/index.html. Data for ages 100+ y for this cohort are not available. Census estimates of the sex ratio of this cohort are available only for ages 0, 10, 20, and 30 y.) These sex ratio estimates are not CSRs because they are defined by age from birth, not by age from conception.

The sex ratio at age 18 y was 0.488 (95% CI: 0.487–0.489, n = 1,843,000). At age 40 y, it was 0.501 (95% CI: 0.500–0.501, n = 1,823,210). At age 60, it was 0.483 (95% CI: 0.482–0.484, n = 1,525,828). If parental expenditure ends at age 40 y, these

data support the prediction of 0.5. This adaptationist conclusion would be more credible if we understood why natural selection has not eliminated the high level of prebirth mortality, especially when it appears to result in no net change in the sex ratio from conception to age 40 y. The failure of three-quarters of conceptions to reach sexual maturity engenders energetic costs, which presumably could be eliminated to the evolutionary benefit of parents. Alternatively, such "screening" could be beneficial to parents. We take no position and stress the need to consider the totality of evidence when making adaptive claims about the human sex ratio and human pregnancy (118–121). We emphasize that our analysis of the 1900 cohort data illustrates how little one conclude about the adaptive significance of the human sex ratio without data on investment, even when the analysis is based on age-specific sex ratio estimates that are among the best available. This ambiguity is an important cautionary lesson, which is underscored by our result that female mortality during pregnancy may be greater than male mortality. All other things being equal, this greater female mortality implies that the sex ratio at investment equilibrium should be male-biased.

The 1900 cohort data can also be compared with the predictions of Charlesworth's (122) model of sex ratio evolution for an age-structured population. His evolutionarily stable strategy model predicts that the PSR is male-biased and that the age-specific sex ratio attains a female-biased equilibrium value (p. 356) by "the end of the first year of postnatal life"; Charlesworth defined parental investment solely as the production of offspring plus the replacement of offspring lost during pregnancy or soon thereafter. As such, his model is at best applied to our primate ancestors or to those human groups and societies in which the human sex ratio might have evolved. Nonetheless, he asserted that his "firm prediction" of a female bias at the "end of infancy" is confirmed in "pre-industrial" societies, although he did not provide sex ratio data. The 1900 cohort exhibits significantly male-biased sex ratios until age 15, which are not consistent with his prediction. This cohort presumably does not qualify as "pre-industrial"; however, sex ratios in hunter–gatherer, horticultural, and pastoral societies are most often similarly male-biased at birth and at age 15 y (123).

Finally, we note that it is not self-evident that the sex ratio trajectory of a human cohort attains any fixed value (apart from sampling error) before only one sex remains. For example, the sex ratio for the 1900 cohort declines throughout life (although not monotonically). Sex ratio estimates are male-biased until age 15 y, after which almost all are between 0.48 and 0.5 until age 61 y. Estimates then become increasingly female-biased and will attain a value of 0.0, because the oldest humans are female (124). Static idealization of a trait can be misleading if dynamic expression is a central component of a trait's evolutionary response to natural selection (125–127). For the 1900 cohort, perhaps the midlife sex ratios ranging from 0.48 to 0.5 can be idealized as a trait that is a target of natural selection. Determining the validity of this static idealization that the ultimate target of natural selection is a single sex ratio (as opposed to the target being, say, an age-specific sequence of sex ratios) will require data on the sex specificity and timing of parental investment, statistical assessment of the age-specific sex ratios to determine whether they are reasonably regarded as age invariant, and a comparison of the predictive accuracy of relevant static and dynamic adaptive models.

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Table S1. Summary of induced abortion studies

Study	Sex ratio	Males	Females	Sexing method
Bochkov and Kostrova (1)	0.489	440	460	C
Bochkov and Kostrova (2)*	0.508	1,525	1,475	C
Boué et al. (3)	0.600	21	14	K
Bowen and Lee (4)	0.714	5	2	K
Bunak (5)	0.611	33	21	M
Csordas et al. (6)	0.560	560	440	C
Evdokimova et al. (7)	0.526	41	37	K
Goldstein et al. (8)	0.376	35	58	C
Golovachev et al. (9)	0.327	16	33	K
Hahnemann (10)	0.500	86	86	K
Hnevkovsky et al. (11)	0.579	378	275	C
Hoshi et al. (12)†	0.455	407	487	K
Jakobovits et al. (13)	0.522	391	358	M
Kajii et al. (14)‡	0.486	530	561	K
Kellokumpu-Lehtinen and Pelliniemi (15)	0.539	297	254	C
Kerr and Rashad (16)	0.533	8	7	K
Klinger and Glasser (17)§	0.506	746	727	K
Kukharenko (18)	0.587	595	419	C
Kukharenko (19)	0.497	349	353	C
Lee and Takano (20)	0.605	848	554	H
Matsunaga et al. (21)	0.514	95	90	C
Matthiessen and Matthiessen (22)	0.580	459	332	M
Mikamo (23)¶	0.518	381	355	C
Momoli and Volet (24)	0.543	69	58	C
Moore and Hyrniuk (25)	0.475	131	145	C
Ohama (26)	0.505	545	534	K
Pogolrzelska (27)	0.531	69	61	C
Sasaki (28)‖	0.469	452	511	K
Schultze (29)	0.700	156	67	C
Serr and Ismajovich (30)	0.624	78	47	C
Stonova and Selezniova (31)	0.615	8	5	K
Suzomori (32)	0.600	6	4	K
Szontagh (33)**	0.550	165	135	C
Szulman (34)	0.733	11	4	K
Thiede and Metcalfe (35)††	0.595	22	15	C, K
Tonomura et al. (36)‡‡	0.534	325	284	K
Tsuji and Nakano (37)	0.477	122	134	K
Vaida (38)	0.579	123	91	C
Yamamoto (39)§§	0.518	570	530	K
Yasuda et al. (40)	0.439	65	83	K
Zhou et al. (41)	0.537	630	542	K

All but two studies assigned fetuses to trimester. Twenty-four studies assigned gestational age in weeks or a narrow range of weeks. In almost all cases, age was based on an estimate of the LMP. C, chromatin; H, histology; K, karyotype; M, morphology.

*Included results from Kostrova (42).

†Probably included results from Hoshi et al. (43).

‡Probably included results from Kajii et al. (44).

§Included results from Klinger et al. (45).

¶Identical to Mikamo (46).

‖Included results from Makino and Sasaki (47), Makino et al. (48, 49), Sasaki et al. (50, 51), Shimba (52), Makino (53), and Makino et al. (54).

**Identical to Szontagh et al. (55).

††Included results from Thiede and Salm (56).

‡‡Included results from Tonomura et al. (57).

§Included results from Yamamoto et al. (58–60).

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Table S2. Week-specific estimates of the CSR based on fetal-death and live-birth data for the United States from 1995 to 2004

Week	Sex ratio	Males	Females
18	0.512	18,162,805	17,335,131
19	0.512	18,149,803	17,325,305
20	0.512	18,133,380	17,311,832
21	0.512	18,115,431	17,296,645
22	0.512	18,096,738	17,280,309
23	0.512	18,075,460	17,261,458
24	0.511	18,052,256	17,240,668
25	0.511	18,026,483	17,217,305
26	0.511	17,997,574	17,191,404
27	0.511	17,958,594	17,157,699
28	0.511	17,912,050	17,117,048
29	0.511	17,850,789	17,062,918
30	0.511	17,769,904	16,991,973
31	0.511	17,655,443	16,892,387
32	0.511	17,484,850	16,745,317
33	0.510	17,200,884	16,498,846
34	0.510	16,736,525	16,095,007
35	0.508	15,925,796	15,394,480
36	0.506	14,362,094	14,035,032
37	0.501	11,273,505	11,245,724
38	0.495	6,934,085	7,076,644
39	0.493	3,238,602	3,334,960
40	0.493	1,298,124	1,337,331
41	0.493	646,232	663,458
42	0.493	330,479	339,250
43	0.493	163,099	167,812
44	0.495	75,062	76,481
45	0.499	28,537	28,674

Week is defined postconception (CA) as determined by LMP.

Table S3. Mixed-effect analyses of the association between the state of individual chromosomes in ART embryos and the CSR

Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight
XY	All	0.505	20,116	341.468	<0.001
	Abnormal	0.999	323	0	>0.999
	Normal	0.498	19,793		
1	All	0.499	20,263	0	0.988
	Abnormal	0.524	452	8.776	0.012
	Normal	0.498	19,811		
2	All	0.498	20,278	0	0.992
	Abnormal	0.510	467	9.750	0.008
	Normal	0.498	19,811		
3	All	0.498	20,068	0	0.992
	Abnormal	0.485	257	9.499	0.008
	Normal	0.498	19,811		
4	All	0.498	20,200	0	0.985
	Abnormal	0.523	389	8.358	0.015
	Normal	0.498	19,811		
5	All	0.498	20,117	0	0.988
	Abnormal	0.524	306	8.823	0.012
	Normal	0.498	19,811		
6	All	0.498	20,108	0	0.992
	Abnormal	0.512	297	9.757	0.008
	Normal	0.498	19,811		
7	All	0.497	20,155	0	0.967
	Abnormal	0.462	344	6.756	0.033
	Normal	0.498	19,811		
8	All	0.498	20,223	0	0.991
	Abnormal	0.480	412	9.404	0.009
	Normal	0.498	19,811		
9	All	0.498	20,229	0	0.991
	Abnormal	0.486	418	9.430	0.009
	Normal	0.498	19,811		
10	All	0.498	20,166	0	0.991
	Abnormal	0.516	355	9.416	0.009
	Normal	0.498	19,811		
11	All	0.498	20,133	0	0.991
	Abnormal	0.478	322	9.445	0.009
	Normal	0.498	19,811		
12	All	0.498	20,026	0	0.992
	Abnormal	0.486	215	9.607	0.008
	Normal	0.498	19,811		
13	All	0.498	20,286	0	0.993
	Abnormal	0.503	475	9.876	0.007
	Normal	0.498	19,811		
14	All	0.499	20,285	0	0.981
	Abnormal	0.522	474	7.868	0.019
	Normal	0.498	19,811		
15	All	0.497	20,607	0	0.961
	Abnormal	0.466	796	6.426	0.039
	Normal	0.498	19,811		
16	All	0.498	21,224	0	0.992
	Abnormal	0.498	1,413	9.764	0.008
	Normal	0.498	19,811		
17	All	0.498	20,103	0	0.990
	Abnormal	0.515	292	9.207	0.010
	Normal	0.498	19,811		
18	All	0.497	20,239	0	0.972
	Abnormal	0.457	448	7.112	0.028
	Normal	0.498	19,811		
19	All	0.499	20,804	0	0.990
	Abnormal	0.509	993	9.183	0.010
	Normal	0.498	19,811		
20	All	0.498	20,190	0	0.977
	Abnormal	0.476	379	7.503	0.023
	Normal	0.498	19,811		

Table S3. Cont.

Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight
21	All	0.499	20,673	0	0.985
	Abnormal	0.516	862	8.373	0.015
	Normal	0.498	19,811		
22	All	0.498	21,096	0	0.990
	Abnormal	0.493	1,285	9.167	0.010
	Normal	0.498	19,811		

All scored chromosomes were normal except the target chromosome, which could be normal or abnormal.

Table S4. Mixed-effect analyses of the association between the overall state of the embryo (Any) or the state of individual chromosomes and the CSR (aCGH data)

		Blastomere				Blastocyst			
Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight	CSR	*N*	ΔAIC	Akaike weight
Any	All	0.484	12,693	0	0.985	0.507	32476	0	0.898
	Abnormal	0.487	9,384	8.367	0.015	0.511	15,974	4.356	0.102
	Normal	0.474	3,310			0.502	16,502		
XY	All	0.484	12,693	504.835	<0.001	0.507	32,476	570.744	<0.001
	Abnormal	0.812	1,103	0	>0.999	0.999	771	0	>0.999
	Normal	0.453	11,590			0.498	31,705		
1	All	0.484	12,693	0	0.983	0.507	32,476	0	0.991
	Abnormal	0.470	1,768	8.103	0.017	0.498	1,204	9.451	0.009
	Normal	0.486	10,925			0.507	31,272		
2	All	0.484	12,693	0	0.982	0.507	32,476	0	0.929
	Abnormal	0.476	1,598	8.013	0.018	0.479	1,258	5.146	0.071
	Normal	0.485	11,095			0.508	31,218		
3	All	0.484	12,693	0	0.990	0.507	32,476	0	0.982
	Abnormal	0.488	1,355	9.247	0.010	0.483	900	7.990	0.018
	Normal	0.483	11,338			0.507	31,576		
4	All	0.484	12,693	0	0.989	0.507	32,476	0	0.985
	Abnormal	0.474	1,376	8.949	0.011	0.496	1,083	8.347	0.015
	Normal	0.485	11,317			0.507	31,393		
5	All	0.484	12,693	0.652	0.419	0.507	32,476	0	0.992
	Abnormal	0.444	1,481	0	0.581	0.498	1,066	9.656	0.008
	Normal	0.489	11,212			0.507	31,410		
6	All	0.484	12,693	0	0.993	0.507	32,476	0	0.966
	Abnormal	0.480	1,382	9.871	0.007	0.485	983	6.714	0.034
	Normal	0.484	11,311			0.507	31,493		
7	All	0.484	12,693	0	0.943	0.507	32,476	0	0.806
	Abnormal	0.459	1,435	5.626	0.057	0.473	1,202	2.849	0.194
	Normal	0.487	11,258			0.508	31,274		
8	All	0.484	12,693	0	0.991	0.507	32,476	0	0.981
	Abnormal	0.489	1,489	9.357	0.009	0.485	1,149	7.859	0.019
	Normal	0.483	11,204			0.507	31,327		
9	All	0.484	12,693	0	0.993	0.507	32,476	0	0.526
	Abnormal	0.485	1,666	9.885	0.007	0.468	1,344	0.210	0.474
	Normal	0.484	11,027			0.508	31,132		
10	All	0.484	12,693	0	0.985	0.507	32,476	0	0.888
	Abnormal	0.484	1,493	8.402	0.015	0.475	1,190	4.131	0.012
	Normal	0.484	11,200			0.508	31,286		
11	All	0.484	12,693	0	0.993	0.507	32,476	0	0.959
	Abnormal	0.483	1,563	9.983	0.007	0.485	1,185	6.281	0.041
	Normal	0.484	11,130			0.507	31,291		
12	All	0.484	12,693	0	0.992	0.507	32,476	0	0.981
	Abnormal	0.484	1,470	9.653	0.008	0.489	890	7.837	0.019
	Normal	0.484	11,223			0.507	31,586		
13	All	0.484	12,693	0	0.992	0.507	32,476	0	0.963
	Abnormal	0.479	1,683	9.681	0.008	0.486	1,450	6.537	0.037
	Normal	0.485	11,010			0.508	31,026		
14	All	0.484	12,693	0	0.988	0.507	32,476	0	0.986
	Abnormal	0.477	1,729	8.788	0.012	0.494	1,349	8.495	0.014
	Normal	0.485	10,964			0.507	31,127		
15	All	0.484	12,693	0	0.986	0.507	32,476	0	0.990
	Abnormal	0.479	2,047	8.537	0.014	0.500	2,162	9.126	0.010
	Normal	0.485	10,646			0.507	30,314		
16	All	0.484	12,692	0	0.990	0.507	32,476	0	0.969
	Abnormal	0.477	2,428	9.206	0.010	0.513	2,759	6.872	0.031
	Normal	0.485	10,265			0.506	29,717		
17	All	0.484	12,693	0	0.990	0.507	32,476	0	0.979
	Abnormal	0.474	1,674	9.092	0.010	0.488	1,081	7.643	0.021
	Normal	0.485	11,019			0.507	31,395		
18	All	0.484	12,693	0	0.987	0.507	32,476	0	0.755
	Abnormal	0.487	1,682	8.627	0.013	0.473	1,486	2.252	0.245
	Normal	0.483	11,011			0.508	30,990		

Table S4. Cont.

		Blastomere				Blastocyst			
Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight	CSR	*N*	ΔAIC	Akaike weight
19	All	0.484	12,693	0	0.993	0.507	32,476	0	0.993
	Abnormal	0.483	2,620	9.966	0.007	0.503	1,879	9.844	0.007
	Normal	0.484	10,073			0.507	30,597		
20	All	0.484	12,693	0	0.993	0.507	32,476	0	0.949
	Abnormal	0.487	1,787	9.854	0.007	0.484	1,426	5.846	0.051
	Normal	0.483	10,906			0.508	31,050		
21	All	0.484	12,693	0	0.993	0.507	32,476	0	0.983
	Abnormal	0.483	2,026	9.873	0.007	0.506	2,336	8.076	0.017
	Normal	0.484	10,667			0.507	30,140		
22	All	0.484	12,693	0	0.952	0.507	32,476	0	0.872
	Abnormal	0.469	2,184	5.976	0.048	0.488	2,914	3.837	0.128
	Normal	0.487	10,509			0.509	29,562		

Table S5. Mixed-effect analyses of the association between the overall state of the embryo (Any) or the state of individual chromosomes and the CSR) for blastomeres (FISH only) and blastocysts (aCGH)

		Blastomere				Blastocyst			
Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight	CSR	*N*	ΔAIC	Akaike weight
Any	All	0.503	94,535	31.275	<0.001	0.507	32,476	0	0.898
	Abnormal	0.511	59,524	0	>0.999	0.511	15,974	4.356	0.102
	Normal	0.490	35,011			0.502	16,502		
XY	All	0.503	94,535	533.156	<0.001	0.507	32,476	570.744	<0.001
	Abnormal	0.589	16,282	0	>0.999	0.999	771	0	>0.999
	Normal	0.486	78,253			0.498	31,705		
1	All	—	—	—	—	0.507	32,476	0	0.991
	Abnormal	—	—	—	—	0.498	1,204	9.451	0.009
	Normal	—	—	—	—	0.507	31,272		
2	All	—	—	—	—	0.507	32,476	0	0.929
	Abnormal	—	—	—	—	0.479	1,258	5.146	0.071
	Normal	—	—	—	—	0.508	31,218		
3	All	—	—	—	—	0.507	32,476	0	0.982
	Abnormal	—	—	—	—	0.483	900	7.990	0.018
	Normal	—	—	—	—	0.507	31,576		
4	All	—	—	—	—	0.507	32,476	0	0.985
	Abnormal	—	—	—	—	0.496	1,083	8.347	0.015
	Normal	—	—	—	—	0.507	31,393		
	All	—	—	—	—	0.507	32,476	0	0.992
	Abnormal	—	—	—	—	0.498	1,066	9.656	0.008
	Normal	—	—	—	—	0.507	31,410		
6	All	—	—	—	—	0.507	32476	0	0.966
	Abnormal	—	—	—	—	0.485	983	6.714	0.034
	Normal	—	—	—	—	0.507	31,493		
7	All	—	—	—	—	0.507	32,476	0	0.806
	Abnormal	—	—	—	—	0.473	1,202	2.849	0.194
	Normal	—	—	—	—	0.508	31,274		
8	All	0.505	22,113	0	0.984	0.507	32,476	0	0.981
	Abnormal	0.503	4,119	8.274	0.016	0.485	1,149	7.859	0.019
	Normal	0.506	17,994			0.507	31,327		
9	All	0.524	3,678	0	0.947	0.507	32,476	0	0.526
	Abnormal	0.516	655	5.780	0.053	0.468	1,344	0.210	0.474
	Normal	0.526	3,023			0.508	31,132		
10	All	—	—	—	—	0.507	32,476	0	0.888
	Abnormal	—	—	—	—	0.475	1,190	4.131	0.012
	Normal	—	—	—	—	0.508	31,286		
11	All	—	—	—	—	0.507	32,476	0	0.959
	Abnormal	—	—	—	—	0.485	1,185	6.281	0.041
	Normal	—	—	—	—	0.507	31,291		
12	All	—	—	—	—	0.507	32,476	0	0.981
	Abnormal	—	—	—	—	0.489	890	7.837	0.019
	Normal	—	—	—	—	0.507	31,586		
13	All	0.503	89,263	0	0.976	0.507	32,476	0	0.963
	Abnormal	0.505	23,598	12.075	0.024	0.486	1,450	6.537	0.037
	Normal	0.503	65,665			0.508	31,026		
14	All	0.503	18,378	0	0.992	0.507	32,476	0	0.986
	Abnormal	0.500	4,727	9.542	0.008	0.494	1,349	8.495	0.014
	Normal	0.504	13,651			0.507	31,127		
15	All	0.500	78,437	42.555	<0.001	0.507	32,476	0	0.990
	Abnormal	0.518	24,120	0	>0.999	0.500	2,162	9.126	0.010
	Normal	0.492	54,317			0.507	30,314		
16	All	0.504	79,589	0	0.881	0.507	32,476	0	0.969
	Abnormal	0.508	24,097	7.213	0.119	0.513	2,759	6.872	0.031
	Normal	0.502	55,492			0.506	29,717		
17	All	0.502	76,327	9.821	0.007	0.507	32,476	0	0.979
	Abnormal	0.517	18,489	0	0.993	0.488	1,081	7.643	0.021
	Normal	0.498	57,838			0.507	31,395		
18	All	0.503	88,607	0	0.796	0.507	32,476	0	0.755
	Abnormal	0.510	23,587	2.717	0.204	0.473	1,486	2.252	0.245
	Normal	0.500	65,020			0.508	30,990		

Table S5. Cont.

		Blastomere				Blastocyst			
Chromosome	Embryos	CSR	*N*	ΔAIC	Akaike weight	CSR	*N*	ΔAIC	Akaike weight
19	All	—	—	—	—	0.507	32,476	0	0.993
	Abnormal	—	—	—	—	0.503	1,879	9.844	0.007
	Normal	—	—	—	—	0.507	30,597		
20	All	0.502	17,866	0	0.969	0.507	32,476	0	0.949
	Abnormal	0.497	4,896	6.910	0.031	0.484	1,426	5.846	0.051
	Normal	0.504	12,970			0.508	31,050		
21	All	0.503	89,669	0	0.973	0.507	32,476	0	0.983
	Abnormal	0.510	25,434	7.151	0.027	0.506	2,336	8.076	0.017
	Normal	0.500	64,235			0.507	30,140		
22	All	0.504	80,548	0	0.992	0.507	32,476	0	0.872
	Abnormal	0.503	25,218	9.567	0.008	0.488	2,914	3.837	0.128
	Normal	0.504	55,330			0.509	29,562		

Table S6. Birth sex ratios for ART conceptions and for natural conceptions in Australia and New Zealand between 1979 and 2011

	ART			Natural		
Year	Sex ratio	Males	Females	Sex ratio	Males	Females
1991	0.516*	3,554	3,329	0.516	128,738	120,972
1992	0.528	702	628	0.514	134,317	126,961
1993	0.529	807	719	0.515	133,289	125,480
1994	0.515	1,029	968	0.515	133,525	125,583
1995	0.498	1,216	1,226	0.514	132,492	125,031
1996	0.514	1,416	1,340	0.515	130,967	123,279
1997	0.523	1,993	1,815	0.514	129,614	122,708
1998	0.521	2,174	1,999	0.513	128,928	122,340
1999	0.516	2,443	2,287	0.513	129,714	122,913
2000	0.512	2,699	2,571	0.514	129,407	122,502
2001				0.514	130,647	123,581
2002	0.511	3,543	3,386	0.513	127,263	120,788
2003	0.506	3,836	3,739	0.515	128,375	120,867
2004	0.509	4,022	3,887	0.515	128,307	120,918
2005	0.512	4,745	4,515	0.513	134,047	127,035
2006	0.507	5,091	4,942	0.516	139,208	130,733
2007	0.510	5,580	5,362	0.514	144,397	136,630
2008	0.513	5,952	5,661	0.514	145,444	137,641
2009	0.521	6,814	6,256	0.514	145,786	137,705
2010	0.521	6,263	5,756	0.511	145,807	139,401
2011	0.521	6,446	5,936	0.514	147,489	139,638
Total	0.515	70,325	66,322	0.514	2,827,761	2,672,706

*For 1979–1991.

Table S7. Birth sex ratios of babies born via by GIFT in Australia and New Zealand between 1985 and 2011

Year	Sex ratio	Males	Females
1985–1991	0.516	2,003	1,881
1992	0.535	549	477
1993	0.518	524	487
1994	0.527	457	410
1995	0.506	325	317
1996	0.544	357	299
1997	0.522	236	216
1998	0.512	148	141
1999	0.504	116	114
2000–2001	0.529	119	106
2002	—	—	
2003	—	—	
2004	0.567	17	13
2005	—	—	
2006	—	—	
2007	—	—	
2008	—	—	
2009	—	—	
2010	—	—	
2011	—	—	
Total	0.521	4,851	4,461

—, no data.

Table S8. PSR estimates from mammals

Species and study	Sex ratio	Males	Females	Sexing method
Cat; Graham (1954) (1)	0.450	9	11	NM
Cat; Austin and Amoroso (1957) (2)	0.483	14	15	NM
Hamster; Sundell (1962) (3)	0.643	63	35	NM
Hamster; Chow et al. (1996) (4)	0.531	51	45	NM
Mouse; Macdowell and Lord (1925, 1926) (5, 6)	0.501	416	415	NM
Mouse; Vickers (1967) (7)	0.500	49	49	NM
Pig; Crew (1925) (8)	0.576	592	436	NM
Pig; Parkes (1925) (9)	0.591	166	115	NM
Pig; Axelson (1968) (10)	0.542	13	11	NM
Rabbit; Melander (1962) (11)	0.509	28	27	NM
Rabbit; Fechheimer and Beatty (1974) (12)	0.486	211	223	NM
Roe Deer; Aitken (1974) (13)	0.514	18	17	NM
Sheep; Henning (1939) (14)	0.509	495	477	NM
Cat; Ciani et al. (2008) (15)	0.568	21	16	M
Cow; Utsumi and Iritani (1993) (16)	0.488	21	22	M
Cow; Hasler et al. (2002) (17)	0.492	1,950	2,014	M
Mouse; Bradbury et al. (1990) (18)	0.558	48	38	M
Mouse; Kunieda et al. (1992) (19)	0.479	34	37	M
Mouse; Byrne et al. (2006) (20)	0.514	247	234	M
Pig; Pomp et al. (1995) (21)	0.536	112	97	M
Sheep; Catt et al. (1997) (22)	0.592	45	31	M
Sheep; Gutiérrez-Adán et al. (1997) (23)	0.500	18	18	M
Sheep; Green et al. (2008) (24)	0.381	8	13	M

M, molecular; NM, nonmolecular.

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Table S9. Age-specific estimates of the sex ratio of the 1900 cohort in the United States

Age, y	Sex ratio	Male	Female	Age, y	Sex ratio	Male	Female	Age, y	Sex ratio	Male	Female
0	0.507	919,000	892,000	35	0.499	919,828	923,875	70	0.430	546,846	725,128
1	0.506	945,000	924,000	36	0.499	917,682	920,743	71	0.426	521,292	702,415
2	0.505	964,000	946,000	37	0.499	915,175	917,354	72	0.420	489,586	675,115
3	0.504	972,000	955,000	38	0.500	913,475	914,880	73	0.415	464,833	655,005
4	0.504	974,000	959,000	39	0.500	911,200	912,647	74	0.408	434,255	631,109
5	0.504	972,000	957,000	40	0.501	912,568	910,642	75	0.400	405,468	608,280
6	0.504	965,000	949,000	41	0.501	912,038	909,471	76	0.392	386,492	599,081
7	0.504	956,000	940,000	42	0.501	910,391	907,147	77	0.384	362,430	582,115
8	0.505	949,000	931,000	43	0.502	910,601	904,809	78	0.382	356,824	578,417
9	0.505	944,000	925,000	44	0.502	909,509	902,868	79	0.373	321,181	538,944
10	0.506	944,000	923,000	45	0.501	910,867	906,472	80	0.361	262,589	465,269
11	0.507	946,000	921,000	46	0.501	906,441	903,237	81	0.350	231,064	429,714
12	0.506	951,000	927,000	47	0.501	898,724	896,378	82	0.346	208,777	395,048
13	0.505	960,000	941,000	48	0.500	887,369	886,839	83	0.336	192,055	378,789
14	0.502	964,000	955,000	49	0.500	874,468	875,479	84	0.326	172,718	356,564
15	0.501	959,000	957,000	50	0.499	863,972	866,456	85	0.317	150,549	323,731
16	0.498	945,000	951,000	51	0.498	865,284	871,306	86	0.308	129,315	290,007
17	0.497	931,000	944,000	52	0.498	854,858	861,998	87	0.299	110,707	259,976
18	0.488	899,000	944,000	53	0.497	831,596	840,521	88	0.289	90,412	222,118
19	0.487	892,000	941,000	54	0.497	816,115	827,159	89	0.275	81,234	214,677
20	0.492	912,000	943,000	55	0.495	810,175	825,897	90	0.262	61,358	172,487
21	0.492	912,000	943,000	56	0.494	799,549	820,515	91	0.251	50,066	149,463
22	0.491	909,000	944,000	57	0.492	793,459	820,901	92	0.240	40,219	127,244
23	0.494	931,000	954,000	58	0.492	803,724	829,370	93	0.228	31,483	106,462
24	0.496	949,000	963,000	59	0.486	766,040	809,007	94	0.219	24,115	86,082
25	0.496	941,000	955,000	60	0.483	736,335	789,493	95	0.209	17,463	66,114
26	0.496	929,000	944,000	61	0.479	708,734	769,803	96	0.198	12,925	52,319
27	0.496	929,000	943,000	62	0.476	686,775	755,702	97	0.191	9,385	39,726
28	0.497	939,000	950,000	63	0.472	669,899	749,115	98	0.184	6,576	29,139
29	0.497	939,000	951,000	64	0.467	656,218	747,776	99	0.189	4,616	19,840
30	0.497	929,367	939,650	65	0.462	641,224	745,983				
31	0.498	927,343	936,201	66	0.456	624,057	744,682				
32	0.498	924,892	932,409	67	0.450	606,110	740,306				
33	0.498	922,718	928,996	68	0.445	583,782	728,696				
34	0.499	921,325	926,446	69	0.440	557,079	709,467				