The Human Female Paradox: Biological Disadvantage Preimplantation, Biological Advantage, Thereafter

Migeon BR*

The Department of Genetic Medicine and Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

*Correspondence: Barbara R Migeon, McKusick-Nathans Department of Genetic Medicine, 413 Miller Research Building, 733 N. Broadway, Baltimore MD 21205, Maryland, United States, E-mail: bmigeon@jhmi.edu

Received date: December 15, 2020; Accepted date: January 26, 2021; Published date: January 31, 2021

The vital statistics show that human females outlive males at every biological stage. Once the embryo arrives in the uterus, more males die at every stage, at least until the eighth decade when the majority of survivors are female. Unexpectedly, the same statistics also show that more boys are born than girls, which is difficult to explain, because the sperm that determine the sex of the fetus, are not skewed toward males. Recently, new data reveal the reason for the increased number of male births; they imply the significant loss of females before the fetus arrives in the womb. Thereafter, there is an excessive loss of males – not only in utero but throughout their lives. One likely reason for the sex differences in fetal survival is the way that humans compensate for the sex difference in number of X chromosomes.

The sex differences in human disease and survival have been attributed to sex differences in hormones and life experiences. However, we now know that many sex differences are due to the unequal numbers of X chromosomes in males and females [1,2]. Human males have 46 chromosomes that include 22 pairs of autosomes, and an XY pair of sex chromosomes. Females also have 46 chromosomes with the same autosomes, but XX sex chromosomes. Therefore, although ~95% of the human genome is common to the two sexes, males and females differ in the number and kinds of genes on their sex chromosomes.

The genetic difference between the sexes is large because the Y chromosome has less than 100 genes, whereas the X has more than 1000 genes, and because females have two gene-rich X chromosomes [3]. Furthermore, unlike the Y genes, which mainly specify the male sex determinants, only a few of the 1000 X-linked genes are concerned with female sex determination. Most of them specify the cellular make-up of our non-sexual tissues and organs, by manufacturing the proteins that are needed for our organs to function; in fact, most genes on the X chromosome resemble autosomal genes.

We know that most X-linked genes affect non-sexual organs and tissues because when mutated, they cause hemophilia, anemia and alpha thalassemia (diseases of blood cells), muscular dystrophy, spinal muscular atrophy, severe immunodeficiency, intellectual deficiency, congenital epilepsy, spina bifida, ichthyosis, rickets, kidney disease, congenital heart disease, deafness, macular degeneration and other eye diseases, congenital malformations of the face, adrenoleukodystrophy and many other disorders, not involving the sex organs [4].

Also, we know that mechanisms have evolved to compensate for the sex difference in the number of X chromosomes, because females who transcribe the genes on both of their X chromosomes die in utero; they are never seen among
live-born populations. In fact, human females are malformed, and retarded, when they express only a minute piece of their second X chromosome [5]. We also know that the transcription of X chromosomes is regulated in both males and females in order to equalize the RNA output from the X chromosomes [6]. This process, which is called X chromosome dosage compensation, occurs during embryogenesis in every species with an XX/XY sex determining mechanism [7].

The details of the compensatory process differ from species to species because of differences in the staging of embryogenesis, and evolutionary changes to the blueprint of the relevant genes [8]. On one hand, flies compensate by increasing the transcription of the X in males so that it equals the two X's in a female; on the other hand, in mammals, all but one X chromosomes is silenced in each cell, regardless of its parental origin [7]. The mammalian method of dosage compensation is called X inactivation, a term initiated by Mary Lyon who was the first to suggest that only a single X was expressed in each cell of female mammals; any X chromosome in addition to one is silenced in males as well as females [9].

Although a female has only one working X in each of her cells, she differs from most males, because she is mosaic for the X chromosome that is expressed; that is, which of her two X chromosome is the active one, differs from cell to cell [10]. The process of X-silencing happens during early embryogenesis – about the time of implantation, when gastrulation occurs in the human fetus. This means that unlike males, who have the same X chromosome in each of their cells, (the one inherited from their mother), females have some cells, which express her paternal X, as well as other cells, which transcribe her maternal X.

Having two kinds of cells would be meaningless, if the genes on the human maternal X did not differ from those on the paternal X. However, recent studies show that the two sets of genes are often dissimilar because of chance mutations that have occurred, which alter the function of the proteins that they encode [4,11].

Also, the genes on all chromosomes, except the sex chromosomes, come in pairs, (we have two copies of each autosome, one derived from our mother, the other from our father). Having two copies of a gene usually protects us from the effect of mutations that interfere with their function; if one copy of a gene is disabled, then the other copy of that gene still retains its function [11]. Fortunately, 50% of normal gene activity often provides enough gene products. However, for males with the same X chromosome in every cell, the effect of mutations in any of its 1000 genes can be devastating [11,12].

Unlike males, the same mutations in genes on the active X chromosome of females affect only some of their cells, because the normal copy on her second X chromosome is expressed in the other cells [12].

The female advantage is that gene products in the cell that can synthesize them often are transferred to the defective cells; the have-cells with a normal amount of gene product share their product with the have-nots – by means of blood, gap junctions, and other means of intercellular communication. When sharing cannot occur, then the defective cells often divide less often, and they are overgrown by the normal cell [12]. Product-sharing and cell selection convey a tremendous advantage to females of our species. With some rare exceptions, only males have hemophilia, or Duchenne muscular dystrophy, and many other X-linked diseases. The only X-linked diseases invariably affecting females are those that are lethal to the male fetus [12].

The vital statistics tell us that having the same X chromosome in every cell is hazardous for males, contributing to their excessive loss at every stage of life after implantation (Table 1 and 2). Only after age 75 years does the ratio of male to female deaths become equal (Table 2); thereafter, more females die than males because by that time, most males have already died.

Because more males are lost after implantation, it was difficult to understand why more males are born than females; the ratio of M: F births are ~1.05, based on many years of vital statistics [13] see table 3. This sex bias in births is the same for most nations in the United Nations that record such data [13]. As skewing of numbers of Y bearing sperm does not occur [14,15], there has been no compelling explanation for the excess of male births – until recently.
Table 1: Infant mortality: United States in 2018 [including M:F Sex ratio in 2013].

| Number of deaths       | Male | Female | M: F Sex ratio 2018 | M:F Sex ratio 2013 |
|------------------------|------|--------|---------------------|-------------------|
| Less than 1 day        | 5,226| 4,253  | 1.23                | 1.24              |
| 1-6 days               | 1,733| 1,355  | 1.28                | 1.3               |
| 7-27 days              | 1,632| 1,453  | 1.12                | 1.22              |
| 28 days -11 months     | 4,417| 3,386  | 1.3                 | 1.33              |
| Total                  | 13,008| 10,447| 1.36                | 1.27              |

Table 2: Sex ratio for mortality at various ages in the United States.

| Age in years | 2008a | 2014a | 2018a |
|--------------|-------|-------|-------|
|              | M:F   | M:F   | M:F   |
| 0-1          | 1.26  | 1.25  | 1.33  |
| 1-4          | 1.32  | 1.3   |       |
| 5-14         | 1.39  | 1.5   |       |
| 15-24        | 2.77  | 2.6   | 1.24  |
| 25-34        | 2.26  | 2.25  | 1.05  |
| 35-44        | 1.68  | 1.6   | 1.13  |
| 45-54        | 1.67  | 1.53  |       |
| 55-64        | 1.48  | 1.55  |       |
| 65-74        | 1.26  | 1.32  |       |
| 75-84        | 0.95  | 1.02  |       |
| >85          | 0.51  | 0.81  |       |

a year data collected
https://unstats.un.org/unsd/demographic/products/dyb/dyb2014.htm Table 16
Latest available data 2018a (Reporting data from 2015 only, and less age-groups were reported.)

Table 3: Sex ratio at birth at various United States in 2013.

| Maternal age in years | Number of male births | Number of female births | M:F Sex ratio |
|-----------------------|-----------------------|-------------------------|---------------|
| 0 - 14                | 3,098                 | 1,592                   | 1.95          |
| 15 - 19               | 140,110               | 132,995                 | 1.05          |
| 20 - 24               | 458,528               | 438,217                 | 1.05          |
| 25 - 29               | 574,266               | 546,511                 | 1.05          |
| 30 - 34               | 530,707               | 506,220                 | 1.05          |
| 35 - 39               | 247,753               | 236,120                 | 1.05          |
| 40 - 44               | 55,858                | 53,626                  | 1.04          |
| 45 - 49               | 3,813                 | 3,682                   | 1.04          |
| 50 - 54               | 327                   | 350                     | 0.93          |
| Total                 | 2,012,914             | 1,919,227               | 1.05          |

https://unstats.un.org/unsd/demographic/products/dyb/dyb2014.htm Table10
Latest available data
The novel information, which explains at least some of the excess male births, comes from the DECIPHER database [16] that records the numbers of common population variants, such as chromosomal duplications, deletions and single gene variants in human males and females. Because the precise location of many of our genes is known, chromosomal duplications and deletions can be defined at the level of nucleotide pairs in the DNA; the DECIPHER database enables us to look for sex differences in the incidence of such variations. A recent survey of the whole human genome showed sex differences in the incidence of duplications and deletions all along the chromosomes, involving only one or two genes that may be responsible for sex differences in some diseases [17]. However, on chromosome 19, there is a region about half the size of the short arm of the chromosome (~8 mega bases), in which inherited tandem duplications are seen only in human males [18]. The implied loss of human females is highly significant (p < ~10⁻²³), and specific to tandem duplications, as the incidence of deletions of the same region is essentially the same for the two sexes. These results have been interpreted in light of what is known about how X inactivation is achieved in human fetuses [19].

What we know is that one X-linked gene is responsible for silencing the inactive X chromosome in every cell [20]. This gene, called $XIST$ for X Inactive Specific Transcript, does not code for a protein, but synthesizes an RNA that coats its own X chromosome; once spread on the chromosome, $XIST$ functions to attract epigenetic factors that bring that X towards the nuclear lamina and change its chromatin from active to inactive [21]. Therefore, $XIST$ transcribes a non-coding RNA that silences the very chromosome from which it is expressed.

Based on studies of human aneuploid fetuses, only one X is active in any cell with a diploid karyotype (46 chromosomes), but more than one X is active in human triploid fetuses (69 chromosomes) [22]. In contrast to the single active X in normal individuals, triplication of our genome enables the activity of two X chromosomes in most triploid cells [23]. These observations suggest that at least one of the triplicated genes enables the transcriptional activity of more than one X, by repressing the $XIST$ locus on a second X chromosome, permitting that X to be transcribed as well [23].

Although we know how the inactive X is silenced by its $XIST$ locus – a powerful chromosome inactivator that can silence any chromosome from which it is expressed [24] – we do not yet know what protects the active X in the cells of both sexes from inactivation by its own $XIST$ locus? It seems likely that $XIST$ on the future active X needs to be repressed to keep that chromosome active. Therefore, the two active X's in triploid fetuses suggest that the $XIST$ repressor is encoded by an autosome [23].

A tandem duplication of the $XIST$ repressor gene on one autosome of the pair–resulting in an extra dose of $XIST$ repressor– would not be harmful for human males, as they have only one X chromosome. However, if the putative repressor locus on chromosome 19p were duplicated in human females, the amount of repressor could silence the locus on both X chromosomes, resulting in two active X chromosomes, a known embryonic lethal event [5]. Therefore, duplications of the relevant region would be tolerated by males, but would be lethal to females, with two X chromosomes. The fact that tandem duplications of this region of chromosome 19p are lethal to females but not to males, are consistent with these expectations. That the short arm of chromosome 19 is the only extensive region of the human genome that shows a sex difference with respect to survival of duplications strongly suggests that this is the region that includes the $XIST$ repressor [18]. New data in the Decipher database suggests that the number of 19ps is relevant as only tandem duplications produce lethality [25].

Among the genes in the exceptional region of chromosome 19 are potential $XIST$ repressors [18]. All of them could theoretically repress $XIST$, and all are expressed in human embryos, prior to implantation. Among these candidate repressors are DNA methyltransferase 1, which when deleted in mice induces $Xist$ expression on the active X of males [26], and its co-factor, $UHRF1$, two satellite attachment factors $SAFB$ and $SAFB2$ and a cluster of zinc finger proteins that arrived on chromosome 19 after humans evolved from rodents. In addition, these human genes, which are found on chromosome 19 in all primates, reside on two different chromosomes in rodents, which strongly suggest that the way we repress $XIST$ on the single active X may differ from rodents. Therefore, the mouse models of X inactivation are unlikely to reflect the process in human cells. Unfortunately, the earliest human embryonic stem cells currently...
available may have already undergone this part of the process of X inactivation [27]. In edition human embryonic stem cells need to be rendered more immature and the derivation of less mature stem cells requires the use of transcription factors and other agents; these derived cells may, or may not, reflect what is happening during early human development.

What remains to be done is to demonstrate that candidate genes on chromosome 19 are able to repress \textit{XIST} in preimplantation human embryos. Such studies are still forbidden in our country because of NIH guidelines and some state laws, so they can be carried out only in some states if supported by private funds [28,29]. However, many other countries carry out such studies until the human embryo reaches day 14. In effect, this reflects their appraisal that the embryo before 14 days of development is not the equivalent of a fetus or person and, thus, may be studied. I suggest that it is time to reconsider the United States guidelines for scientific experimentation, and to extend our ability to study human embryos to embryonic day 14, so that we will know the early developmental events that occur in our species. It is likely that mice will not be useful models as they differ from humans in regard to many early developmental events like this one [8].

\textbf{References}

1. Arnold AP, Disteche CM. Sexual inequality in the cancer cell. \textit{Cancer Res.} 2018;78(19):5504-5505. Doi: https://doi.org/10.1158/0008-5472.CAN-18-2219
2. Migeon BR. The role of X inactivation and cellular mosaicism in women's health and sex-specific diseases. \textit{JAMA.} 2006;295(12):1428-1433. Doi: https://doi.org/10.1001/jama.295.12.1428
3. Ross MT, Graffam DV, Coffey AJ, et al. The DNA sequence of the human X chromosome. \textit{Nature.} 2005;434(7031):325-337. Doi: https://doi.org/10.1038/nature03440
4. https://www.ncbi.nlm.nih.gov/omim
5. Migeon BR, Luo S, Jani M, et al. The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation. \textit{Am J Hum Genet.} 1994;55(3):497-504. https://pubmed.ncbi.nlm.nih.gov/8079992/
6. Disteche CM. Dosage compensation of the sex chromosomes and autosomes. \textit{Semin Cell Dev Biol.} 2016;56:9-18. Doi: https://doi.org/10.1016/j.semcdb.2016.04.013
7. Lucchesi JC. Transcriptional modulation of entire chromosomes: Dosage compensation. \textit{J Genet.} 2018;97(2):357-364. Doi: https://doi.org/10.1007/s12041-018-0919-7
8. Migeon BR. An overview of X inactivation based on species differences. \textit{Semin Cell Dev Biol.} 2016;56:111-116. Doi: https://doi.org/10.1016/j.semcdb.2016.01.024
9. Lyon MF. Sex chromatin and gene action in the mammalian X-chromosome. \textit{Am J Hum Genet.} 1962;14(2):135-148. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1932279/
10. Migeon BR. Females are mosaics: X inactivation and sex differences in disease.2016. Doi: https://doi.org/10.1093/med/9780199927531.001.0001.
11. Childs B. Genetic Medicine: A logic of Disease. Baltimore: Johns Hopkins University Press; 1999. https://muse.jhu.edu/article/26077
12. Migeon BR. X-linked diseases: Susceptible females. \textit{Genet Med.} 2020;22(7):1156-1174. Doi: https://doi.org/10.1038/s41436-020-0779-4
13. Mathews Tj, Hamilton BE. Trend analysis of the sex ratio at birth in the United States. \textit{Natl Vital Stat Rep.} 2005;53(20):1-18. https://pubmed.ncbi.nlm.nih.gov/15974501/
14. Boklage CE. The epigenetic environment: Secondary sex ratio depends on differential survival in embryogenesis. \textit{Hum Reprod.} 2005;20(3):583-587. Doi: https://doi.org/10.1093/humrep/deh662
15. Orzack SH, Stubbeifield JW, Akmaev VR, et al. The human sex ratio from conception to birth. \textit{Proc Natl Acad Sci USA.} 2015;112(16):E2102-E2111. Doi: https://doi.org/10.1073/pnas.1416546112
16. Firth HV, Richards SM, Bevan AP, et al. DECIPHER: Database of chromosomal imbalance and phenotype in humans Cur Op Gyn Obs, 3(1): 397-404 (2020)
17. Migeon BR, Beer MA, Bjornsson HT. Embryonic loss of human females with partial trisomy 19 identifies region critical for the single active X. Wutz A, ed. *PLoS ONE*. 2017;12(4):e0170403. Doi: https://doi.org/10.1371/journal.pone.0170403

18. Migeon BR, Beer MA, Bjornsson HT. Embryonic loss of human females with partial trisomy 19 identifies region critical for the single active X. Wutz A, ed. *PLoS ONE*. 2017;12(4):e0170403. Doi: https://doi.org/10.1371/journal.pone.0170403

19. Migeon BR. Choosing the active X: The human version of X inactivation. *Trends Genet*. 2017;33(12):899-909. Doi: https://doi.org/10.1016/j.tig.2017.09.005

20. Brown CJ, Hendrich BD, Rupert JL, et al. The human XIST gene: Analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. *Cell*. 1992;71(3):527-542. Doi: https://doi.org/10.1016/0092-8674(92)90520-M

21. Chen CK, Blanco M, Jackson C, et al. XIST recruits the X chromosome to the nuclear lamina to enable chromosome-wide silencing. *Science*. 2016;354(6311):468-472. Doi: https://doi.org/10.1126/science.aae0047

22. Jacobs PA, Migeon BR. Studies of X-chromosome inactivation in trisomies. *Cytogenet Cell Genet*. 1989;50(2-3):75-77. Doi: https://doi.org/10.1159/000132727

23. Migeon BR, Pappas K, Stetten G, et al. X inactivation in triploidy and trisomy: The search for autosomal transfactors that choose the active X. *Eur J Hum Genet*. 2008;16(2):153-162. Doi: https://doi.org/10.1038/sj.ejhg.5201944

24. Czermiński JT, Lawrence JB. Silencing trisomy 21 with XIST in neural stem cells promotes neuronal differentiation. *Dev Cell*. 2020;52(3):294-308.e3. Doi: https://doi.org/10.1016/j.devcel.2019.12.015

25. Migeon BR. Stochastic gene expression and chromosome interactions in protecting the human active X from silencing by XIST. *Nucleus*. 2021;12(1):1-5. Doi: https://doi.org/10.1080/19491034.2020.1850981

26. Panning B, Jaenisch R. DNA hypomethylation can activate XIST expression and silence X-linked genes. *Genes Dev*. 1996;10(16):1991-2002. Doi: https://doi.org/10.1101/gad.10.16.1991

27. Di Stefano B, Ueda M, Sabri S, et al. Reduced MEK inhibition preserves genomic stability in naïve human embryonic stem cells. *Nat Methods*. 2018;15(9):732-740. Doi: https://doi.org/10.1038/s41592-018-0104-1

28. Wadman M. Trump administration to review human fetal tissue research. *Science*. 2018. Doi: https://doi.org/10.1126/science.aav5328

29. Knoppers BM, et al. The human embryo: Ethical and legal aspects. Vaillancourt C, Lafond J, eds. *Methods Mol Bio*. 2009;550:281-305. Doi: https://doi.org/10.1007/978-1-60327-009-0_18