Rationale for the study of the human sex ratio in population studies of polluted environments

Justificativa para o estudo da razão de masculinidade em seres humanos através de inquéritos populacionais em ambientes poluídos

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Abstract *The human secondary sex ratio remains a subject of substantial interest. The possibility has been raised that environmental chemical exposures have played a role in the changes associated with the sex ratio in a number of countries. The possibility that such an effect may be present is supported at least theoretically by the observation that clomiphene citrate, a drug used in the treatment of infertility with powerful estrogenic and anti-estrogenic properties, has profound effects on the sex ratio resulting in significantly fewer males at birth. Using a model of causality based on the clinical identification of adverse drug effect methodology one may improve the objectivity of the assessment of significant environmental exposures on this human reproductive outcome.*

Key words *Sex Ratio; Reproduction; Infertility; Endocrine Disruptors*

Resumo *A razão secundária de masculinidade em seres humanos continua suscitando bastante interesse. Em diversos países foi levantada a hipótese do papel da exposição química ambiental nas alterações associadas à razão de masculinidade. Tal efeito é sugerido, pelo menos teoricamente, pela observação de que o citrato de clomifene, droga utilizada no tratamento da infertilidade, com potentes propriedades estrogênicas e anti-estrogênicas, tem efeitos profundos sobre a razão de masculinidade, resultando no nascimento de uma proporção significativamente menor de machos. Utilizando um modelo causal baseado na identificação clínica de uma metodologia de efeito farmacológico adverso, pode-se melhorar a objetividade da avaliação da exposição ambiental significativa sobre esse desfecho reprodutivo em seres humanos.*

Palavras-chave *Razão de Masculinidade; Reprodução; Infertilidade; Desreguladores Endócrinos*

Introduction

The secondary sex ratio remains a prominent subject of interest from many perspectives including the controversial position that the ratio may be modified by exposure to environmental chemicals (Davies et al., 1998; James, 2000). Although the ratio was noted to vary significantly over time among populations many years ago, the actual mechanisms of such variation have yet to be identified (Gini, 1908). The sex ratio is defined as the proportion of male births to female births. It includes live birth as well as stillbirths so that a reduction in stillbirths which are predominantly male, can reduce the ratio, through the improvement of prenatal care.

Recent reports have described changes in the sex ratio over varying periods of time in a number of countries. Significant reductions in the ratio, indicating fewer male births, have been identified in some countries, including Canada (Allan et al., 1997), the United States (Allan et al., 1997; Marcus et al., 1998), Denmark (Moller, 1996), Holland (van der Pal-de Bruin, 1997), Germany (van den Broek, 1997) and England and Wales (Manning et al., 1997). Not all countries have identified reductions in the sex ratio. Australia has reported no change in the sex ratio (Lancaster & Day, 1998) while Ireland has reported an increase in the proportion of male births to females over similar time periods (Moynihan & Breathnach, 1999).

Although the effect size of the change in the ratios is very small, these small changes can be statistically significant due to the large sample size of the population under review. For example, in Canada, although the effect of the decline from 1970 to 1990 was estimated to be approximately 8,639 fewer males, the changes were highly statistically significant, despite the fact that this represents only 0.11% of the total of 7,740,324 births for that period of time (Allan et al., 1997).

The trend in sex ratio reduction has been suggested by some to be indicative of potential influences of the environment on reproductive function (Solomon, 2000). This has coincided with observations that there have been secular reductions in the sperm counts of men in addition to increased rates of abnormalities of male genitalia. Some have suggested that the sex ratio be utilized as a sentinel marker for evidence of broad reproductive injury in humans (Davis et al., 1998).

This paper is directed to critically examine the subject of the use of sex ratio as a marker for environmental chemical injury, causing adverse reproductive effects.

Population studies

Potential influences that might explain the variation in the sex ratio among populations are numerous. Proposed determinants have included race, parental age at conception, timing of coitus and/or insemination, associated diseases of the parents and external factors such as warfare and temperature (James, 1987a). It is possible that the many variables that have been identified as significantly associated with the sex ratio may in fact be a function of the substantial power associated with the use of large population sample sizes. Although these variables have been associated with changes in the ratio, it is unclear what potential mechanisms operate to effect the changes. One proposed theory suggests that hormonal status of the parents plays a role in determining the sex of the offspring. Hormonal status could be influenced by the proposed determinants (parental age, disease, etc.) (James, 1987b). Although this mechanism has been proposed, the actual process remains unknown.

More recently, a large scale linkage study that correlated the population sex ratio with family structure has provided important insights into potential mechanisms (Biggar et al., 1999). In Denmark from 1960-1994, a significant reduction in the sex ratio was attributed to several factors. It was noted there was an association with the reduction in family size and preference of sex, functions of choice among the couples involved. Additionally, there appeared to be a differing probability of having males or females within each couple that appeared to be an individual function of each couple that would be independent of choice. This was termed, the biologic heterogeneity of the couple. There is however, no proposed mechanism for the biological heterogeneity that would appear to exist among couples. It should be noted that a study in the same country from 1980-1994 failed to indicate such an effect of biological heterogeneity was ever present (Jacobsen et al., 1999).

Exposure studies

Information relating positive effects of environmental chemicals and the sex ratio has been limited to a number of possible candidates. These have involved small cohorts associated with intense exposures (such as accidental poisonings) to certain chemicals as well as occupational exposure studies. Causality is always difficult to assess due to the complexities of

such exposures. To assist with the question of causality, it is helpful to note that identifying an adverse reaction to toxic exposure relies on similar rigor to the determination of adverse clinical outcomes from therapeutically administered drugs. A modification of the algorithm designed by Kramer et al. (1979) has been used to weigh the evidence of causality (Table 1). Similar to the area of potential adverse drug reaction, the toxic exposure literature is also complex owing to the fact that there may be positive and negative studies and incomplete information regarding the exposure episode or exposure to a mixtures of chemicals. Table 1 has been adapted to adjust the indicator of causality by a measure that reflects the strength of the study while considering studies that show competing results. A scoring system assigns a score relating the degree of certainty that the chemical studied may play a role in exerting an influence on the sex ratio. In this case, the possibility of a change in the sex ratio is considered a possible adverse effect. This algorithm was approached using a simplified approach as defined by Sackett et al. (1985).

The evidence for toxic chemical effects on human sex ratio assigns a certainty score for causality using the modified algorithm from Kramer et al. (1979) and is summarized in Table 2. The potentially toxic chemicals analyzed have been identified in the occupational and accidental exposure literature. For a broader perspective of the weight of evidence of these chemicals and reduction of the sex ratio, a comparison was made to a potent drug with a powerful effect on the sex ratio. This is included in the table. Clomiphene citrate, administered in the treatment of infertility, has a po-

Table 1

A scoring strategy for deciding whether this chemical exposure caused the reported adverse effects.

		Scoring System	
	Add ≥1 to Score	0	Subtract ≥ 1 to Score
Step 1			
Adverse effect established	Adverse effect well accepted as adverse reaction	Adverse effect is not well known or chemical is new	Adverse effect previously unreported as adverse reaction to well known chemical exposure
Step 2			
Alternatives	No good alternative chemical candidate (Score +2)	Chemical candidate(s) exist, but no good ones	Good alternative chemical candidate
Step 3			
Timing	Timing appropriate for adverse reaction to chemical	Timing equivocal or not assessable	Timing inconsistent for adverse reaction to this chemical
Step 4			
Biological evidence	Biological samples provide unequivocal evidence of exposure	Not obtained, unknown, or equivocal evidence of exposure	No biological samples indicate exposure
Step 5			
Diminishes	Adverse effect diminishes after exposure ended	Adverse effect diminishes but incomplete or unexpected rate or degree	Adverse effect diminishes without ending exposure
Step 6			
Recurs	Adverse effect recurs or exacerbates with re-exposure	No re-exposure	Adverse effect fails to recur with re-exposure
Step 7			
Negative study	Ignore if none exists	Ignore if none exists	If negative study exists, subtract one

Certainty Score Score	Degree of Certainty
6 to 7	Definitely did so
4 to 5	Probably did so
0 to 3	Possibly did so
Negative	Unlikely

Adapted from Kramer et al. (1979).

Table 2

Application of causality algorithm to environmental chemical exposures with adverse effects on human sex ratio.

Certainty score	Dioxin[1]	HCB[2]	DBCP[3]	Clomid[4]
Step 1				
Adverse effect established	0	0	0	0
+1				
Step 2				
Alternatives	+2	+2	+2	+2
+2				
Step 3				
Timing	+1	+1	+1	+1
+1				
Step 4				
Biological evidence	+1	+1	0	+1
+1				
Step 5				
Diminishes	+1	0	+1	+1
+1				
Step 6				
Recurs	0	0	0	+1
+1				
Step 7				
Negative study	-1	0	0	-1
-1				
Score	4	4	4	5
Certainty	Probably	Probably	Probably	Probably
References	Mocarelli et al. (1996) Michalek et al. (1998) Landi et al. (1997)	Jarrell et al. (in press)	James (1998) Potashnik (1995)	Jarrell et al. (1993) Dickey et al. (1995) Sampson et al. (1983)

[1] Dioxin or TCDD: 2,3,7,8- tetrachlorodibenzo-p-dioxin; [2] HCB = hexachlorobenzene;
[3] DBCP = dibromochloropropane; [4] Clomid = clomiphene citrate.

tent effect on the sex ratio via unknown mechanisms. This has been noted among patients from a variety of countries (Table 3) and has been analyzed in terms of single and multiple births (Table 4). The mechanism whereby clomiphene citrate reduces the sex ratio is similarly unknown, but may be due to its estrogenic and anti-estrogenic properties through actions on the estrogen receptors of the female. This would be consistent with the ongoing parental hormonal status hypothesis of James as well as the synergistic effects of estrogen on sex determination in the temperature-dependent model of the turtle (Arnold et al., 1997; Bergeron et al., 1999).

Table 2 indicates there is a reasonably strong relationship of reduced sex ratio in association with dioxin (Mocarelli et al., 2000), dibromochloropropane (Potashnik et al., 1995) and hexachlorobenzene (Jarrell et al., in press). The certainty score for causality is similar to that for clomiphene citrate (Jarrell et al., 1993). The observation that hexachlorobenzene, like dioxin, is capable of binding to the Aryl Hydrocarbon Receptor (Ah receptor) albeit with less affinity, suggests the possibility of a similar mechanism, although there is as yet no such determination defined. It is interesting to note, however, that dioxin has been shown to alter the development of early murine embryo development through its action on the Ah receptor (Tsutsumi, 2000) and that Ah receptor agonists are associated with the induction of cytochrome P450 1B1 in normal tissues (Muskhelishvili et al., 2001) and estrogen receptor regulation (Angus et al., 2000). In addition to the adverse effect on sex ratio, hexachlorobenzene is strongly associated with pregnancy loss in

Table 3

Meta analysis results[1] of the effects of clomiphene citrate on human sex ratio.

Study's country	Births	Males	Females	Sex ratio[2]	Z
Canada	20	10	10	100.0	-0.13
Australia	132	73	59	123.7	0.87
United States	100	58	42	138.1	1.30
Israel	88	48	40	120.1	0.57
Israel	22	5	17	29.4	-2.70
Finland	89	41	48	85.4	-1.02
Australia	32	19	13	146.2	0.89
Japan	226	126	100	126.0	1.28
Greece	99	53	46	115.2	0.40
United States	39	13	26	50.0	-2.26
France	57	27	30	90.0	-0.62

[1] Includes studies where the only drug administered was clomiphene citrate; [2] Number of males per 100 females.
Adapted from the meta-analysis for the Royal Commission on New Reproductive Technologies, 1993 (Jarrel et al., 1993).

Table 4

Effect of clomiphene citrate on sex ratio by birth outcome.

Type of Birth	Births	Singletons		Twins		Triplets or higher		All		Sex ratio	Z
		M	F	M	F	M	F	M	F		
All births	2,414							1,171	1,243	94.2	-2.94 [1]
Singletons	222	98	124							79.0	-2.19 [2]
Twins	125			55	70					78.6	-1.68
Triplets	103					49	54			90.7	-0.80

[1] Significant at $p < 0.01$ (two-sided); 2 Significant at $p < 0.05$ (two-sided).
Taken from the meta analysis for the Royal Commission on New Reproductive Technologies, 1993 (Jarrel et al., 1993).

women and severe oocyte toxicity in primates (Jarrell et al., 1998).

Summary

The factors which determine sex of humans at conception is currently unknown. The actual mechanism(s) responsible for variations in the population sex ratio should be clarified before making conclusions regarding external environmental contributions to changes in the population sex ratio. However, in the absence of knowledge regarding mechanisms, evaluation of the association of the sex ratio to other important variables may be helpful. This is particularly useful in large cohort studies where there is reasonable evidence that the ratio can be altered by external agents, including drugs and chemicals which impact the endocrine system. Monitoring the sex ratio in relation to environmental exposures may be beneficial in providing additional insights into the possible mechanism of sex determination. Use of a certainty algorithm for determining causality, derived from the clinical theurapeutics model, strengthens the weight of evidence by applying rigorous constraints to the existing literature.

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