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Is Human Spermatogenesis Uniquely Poor?

Contents

Abstract

We have compiled a list of several parameters of testicular function and efficiency in an attempt to place human male reproduction in a comparative context. Humans are the worst or next to worst in 13 of 15 measures compared with other well-studied mammals. Low human male fecundity may have emerged as an inevitable trade-off during the evolution of life-cycle trends such as longevity, and may even reflect a long-term trend among the higher Primates. Future research to test this hypothesis should concentrate on critical genes on both the X and Y chromosomes that control testicular function and sperm quality, as well as genes and retroviral elements likely to influence the genetic pathways determining testicular function and male reproductive performance.

Key words: Spermatogenesis, infertility, gonadosomatic index, evolution, human reproduction, disposable soma.

This Invited Review is dedicated to the memory of Dr. Lonnie D. Russell.

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Why Re-evaluate Human Spermatogenesis?

There are several reasons why a re-evaluation of human spermatogenesis is timely. The relatively low output of sperm from the human testis was well described nearly 20 years ago (Amann & Howards, 1980). However, today's society is increasingly vigilant about the environment and we are now alert to the potential impact of environmental factors such as xenoendocrine chemicals on human reproduction and health (Kimmel, 1996; Sharpe, 2000). The argument is often posed that, since human male fertility may already be borderline, exposure to such hazards might be sufficient to "tip the scale" towards reproductive incompetence. The ongoing vigorous debate about the "declining sperm count" highlights this concern (Swan *et al.*, 2000). However, an increasing awareness of the complex interaction between disease and evolutionary forces is leading us to revise our perspective on what is "normal" in human health, and evolutionary biology is coming to be recognized as "a basic science essential for medicine" (Nesse & Williams, 1998). In this review, we examine the quantitative state of human spermatogenesis in comparison with other mammalian species that have been well characterized. This is admittedly an imperfect comparison, but we hope it forms a starting point for more rigorous comparative and evolutionary studies.

Comparisons of Testicular Sperm Production Parameters

The results collated from the literature are presented in Tables 1 to 6. We stress that the comparisons must be viewed cautiously, as there are many factors that affect testicular and epididymal function in mammals. Moreover, those species that have indeed been studied in detail are not representative of mammals in general, being restricted to primates, to rodents and the rabbit, and to domestic species that may have been subject to artificial selection for fertility.

All else being equal, the simple size (weight) of the testis lends itself to greater sperm production (Table 1). In general, the species with the largest testis-to-body weight ratio ("gonadosomatic index") are also the "best" sperm producers in terms of quantity per gram of testis (Tables 1 and 5). Testis size has undoubtedly evolved in each species in response to a variety of other factors, beyond the first-order influence of body size. Small animals must allocate a greater proportion of body mass and energy expenditure to testicular maintenance than larger animals (Kenagy & Trombaluk, 1986). Thus, the gonadosomatic index of rats and mice is ten times greater than for humans (Table 1). Some species devote greater relative testicular space to the Leydig cell compartment, for steroidogenesis. This may be caused by varying requirements for testosterone for sperm production and for the maintenance of peripheral androgen levels. In general, it would appear that the larger the animal, the more testosterone is needed peripherally to maintain a given blood concentration. On the other hand, the requirement for sperm numbers is more or less constant or, at least, is not so dependent on the size of the animal. The relative size of the compartment devoted to Leydig cells is therefore variable in different species. Fawcett *et al.* (1973) also noted the extreme variation in relative amounts of interstitial tissue between species and stated "the unusual abundance of interstitial tissue may be related to synthesis of steroid products other than testosterone-pheromones or substances with some other functions as yet undefined".

The relative mass of seminiferous tissue *per se* determines how much space is devoted to sperm production. In general, species where the testis has a high proportion of seminiferous tissue produce more sperm per unit mass (Tables 1 and 5).

The presence of Sertoli cells is essential for sperm production (Russell &

Table 1. Testis weight and seminiferous tubule occupancy.

Species	Testis weight (g)	Relative rank[1]	Gonadosomatic index[2] (%)	Relative rank	Seminiferous tubule (V_V%)	Relative rank[3]	References
Primates							
Man							
Homo sapiens	16.6	5	0.08	8	61.6	7	Johnson *et al.*, 1980
Gorilla							
Gorilla gorilla	11.6	6	0.02	10			Hall-Craggs, 1962
Chimpanzee							
Pongo pygmaeus			0.056	9			Dahl, *et al.*, 1993
Rhesus							
Macaca mullata	24.5	3	0.73	4			Amann *et al.*, 1976
Cynomulgus							
Macaca fascicularis	17.5	4	0.75	3	82.5	5	Zhengwei *et al.*, 1997
Rodentia							
Rat							Russell & França, 1995;
Rattus norvegicus	1.65	8	0.8	1	85.8	2	Wang *et al.*, 1993
Mouse							Bartke *et al.*, 1974;
Mus domesticus	0.1	9	0.78	2	84.5	3	Russell, *et al.*, 1990
Artiodactyla							
Bull							
Bos taurus	402	1	0.1	7	72.5	6	Amann, 1962
Boar							França, 1991;
Sus scrofa	365	2	0.4	5	82.9	4	Swierstra, 1970
Lagomorpha							
Rabbit							
Oryctolagus cuniculus	3.1	7	0.21	6	86.8	1	Amann, 1970

[1] Assumption: the larger the testis, the greater the sperm production.
[2] The "gonadosomatic index" is defined as combined testis weight as a ratio of body weight and, except for the boar (França, 1991), all data are from Kenagy & Trombulak (1986).
[3] Assumption: the greater the percentage volume of seminiferous tubule, the greater the sperm production.

Table 2. Sertoli cell parameters.

Species	Relative volume of Sertoli cells in the epithelium (V_V%)	Relative rank[1]	Number of Sertoli cells/gram of testis ($x10^6$)	Relative rank[2]	Number of Spermatids/ Sertoli cells	Relative rank[3]	References
Primates							
Man							Russell, *et al.*, 1990;
Homo sapiens	35.5 – 40.2	8	48.8	7	3.0	8	Sinha Hikim *et al.*, 1985
Rhesus							
Macaca mullata	24.0 – 32.0	5					Cavicchia & Dym, 1977
Cynomulgus							Russell, *et al.*, 1990;
Macaca fascicularis	31.4 – 31.6	6	32.3	4	12.4	1	Zhengwei, *et al.*, 1997
Rodents							Russell & Defranca,
Rat							1995; Russell &
Rattus norvegicus	19.4 – 26.7	3	40.0 – 41.3	6	10.3	3	Peterson, 1984
Mouse							Croft & Bartke, 1976;
Mus domesticus	12.0 – 16.0	1	36.2	5	7.2	5	Russell, *et al.*, 1990
Artiodactyla							Berndtson & Igboeli,
Bull							1989; 1987; Wrobel &
Bos taurus	27.0 – 38.4	6	27.6	3	8.0	4	Schimmel, 1989
Boar							França & Cardoso, 1998
Sus scrofa	26.4		20.0	1	12.4	1	França (unpublished)
Lagomorpha							
Rabbit							Russell & Peterson,
Oryctolagus cuniculus	16.5 - 14.4	2	24.9	2	12.2	2	1984; Russell, *et al.*, 1990

[1] Assumption: there is an inverse relationship between Sertoli cell occupancy and the space available for germ cells.
[2] Assumption: there is a direct relationship between the number of Sertoli cells per gram of testis and the space available for germ cell production.
[3] Assumption: there is a direct relationship between the number of spermatids per Sertoli cell and overall sperm production.

Table 3. Number of putative differentiated spermatogonial generations and germ cell ratios.

Species	Spermatogonial generations[1]	Relative rank[2]	Coefficient of efficiency of mitosis	Relative rank[3]	Meiotic index*	Relative rank[3]	Overall rate of spermatogenesis**	Relative rank[3]	References
Primates									
Man									Clermont, 1972; Sinha
Homo sapiens	2 (Ap, B)	3	2.5 (38)	2	1.3 (68)	8	3.2 (80)	5	Hikim *et al.*, 1985
Rhesus									
Macaca mulatta	5 (A1-2, B1-3)	2							Clermont, 1972
Cynomulgus									Fouquet & Dadoune,
Macaca fascicularis	5 (Ap, B1-4)	2			3.9 (1.5)	1			1986; Zhengwei *et al.*,1997
Stump-tailed macaque									
Macaca arctoides	5 (Ap, B1-4)	2	29 (9.0)	1	2.5 (38)	7			Clermont & Antar, 1973
Rodents									Clermont, 1972;
Rat									Clermont &
Rattus norvegicus	6 (A1-4, In, B)	1	28 (56)	3	3.4 (15)	3	97 (62)	1	Morgentaler, 1955
Mouse									Bartke *et al.*, 1974;
Mus domesticus	6 (A1-4, In, B)	1	24 (63)	5	3.1 (23)	6	65 (75)	4	Clermont, 1972
Artiodactyla									Amann, 1962;
Bull									Hochereau-de Reviers,
Bos taurus	6 (A1-3, In, B1-2)	1	18 (72)	6	3.6 (10)	2	65 (75)	4	1976
Boar									França, 1991;
Sus scrofa	6 (A1-4, In, B)	1	25 (61)	4	3.2 (20)	5	68 (73)	3	Frankenhuis *et al.*, 1982
Lagomorpha									
Rabbit									
Oryctolagus cuniculus	5 (A1-2, In1-2, B)	2	11.8 (63)	5	3.3 (18)	4	39 (69)	2	Castro, 1995

[1]Spermatogonia are usually classified as type A and B and intermediate (In).
[2]Assumption: the more spermatogonial generations, the greater the overall sperm production.
[3]Assumption: the lower the number between parentheses, the more efficient is spermatogenesis.
* Ratio of number of primary spermatocytes per type A_1 spermatogonia. ** Ratio of early round spermatids per primary spermatocyte.
*** Ratio of round spermatids per type A_1 spermatogonia. Numbers in parentheses show the percentage of degenerations based on the theoretical yield.

Table 4. Duration of spermatogenetic events (days).

Species	Cycle	Total of spermatogenesis[1]	Relative rank[2]	Sperm transit time through epididymis (days)	Relative rank[3]	References
Primates						
Man						Amann, 1981; Heller & Clermont, 1963;
Homo sapiens	16.0	64.0	10	1-21; 5.4	5	Rowley *et al.*, 1970
Rhesus						Amann, *et al.*, 1976;
Macaca mullata	10.5	42.0	3	10.5	7	de Rooij *et al.*, 1986
Cynomulgus						
Macaca fascicularis	10.5	42.0	3			Fouquet & Dadoune, 1986
Stump-tailed macaque						
Macaca arctoides	11.6	46.4	6			Clermont & Antar, 1973
Baboon						
Papio anubis	11.0	44.0	5			Chowdhury & Steinberger, 1976
Chimpanzee						Smithwick *et al.*, 1996a;
Pan troglodytus	13.9	55.6	9	2.0	1	Smithwick *et al.*, 1996b
Rodents						
Rat						Clermont & Harvey, 1965;
Rattus norvegicus	12.9	51.6	7	8.4	3	Robb *et al.*, 1978
Mouse						Dadoune & Alfonsi, 1984;
Mus domesticus	8.6	34.4	1	5.0	2	Oakberg, 1956
Artiodactyla						
Bull						Hochereau-de Reviers, 1976; Koefoed-
Bos taurus	13.5	54.0	8	8.0-11.0	6	Johnsen, 1960
Boar						
Sus scrofa	9.0	36.0	2	9.0	4	França & Cardoso, 1998
Lagomorpha						
Rabbit						
Oryctolagus cuniculus	10.9	43.6	4	8.0-10.0	4	Swierstra & Foote, 1965

[1] Based on 4 cycles beginning at A_1 spermatogonia.
[2] Assumption: short spermatogenic cycles allow for greater sperm production.
[3] Assumption: the longer the transit through the epididymis, the greater the delay between sperm production and sperm availability in the ejaculate.

Table 5. Daily sperm production (DSP) and epididymal sperm reserves.

Species	DSP/gram of testis ($x10^6$)	Relative rank[1]	DSP/ testis ($x10^9$)	Relative rank[2]	Epididymal sperm reserve[3] ($x10^9$)	Relative rank[4]	References
Primates							
Man							
Homo sapiens	4.1	7	0.072	5	0.64	6	Amann, 1981
Rhesus							
Macaca mullata	23.3	5	0.55	3	11.1	3	Amann *et al.*, 1976
Rodents							
Rat							
Rattus norvegicus	24.0	4	0.043	6	0.74	5	Robb *et al.*, 1978
Mouse							Joyce *et al.*, 1993;
Mus domesticus	37.6	1	0.003	7	0.08	7	Meistrich *et al.*, 1976
Artiodactyla							
Bull							
Bos taurus	12.0	6	3.8	2	57.0	2	Amann, 1981
Boar							
Sus scrofa	24.1	3	8.2	1	161.2	1	Swierstra, 1970
Lagomorpha							Amann & Lambiase,
Rabbit							1969; Lambiase &
Oryctolagus cuniculus	25.0	2	0.080	4	2.1	4	Amann, 1969

[1]Assumption: the higher the DSP per gram of testis, the most efficient is spermatogenesis.
[2]Assumption: the greater the DSP the more sperm available for use.
[3]Both epididymides included.
[4]Assumption: the greater the epididymal sperm reserves the more sperm available for delivery to the ejaculate.

Table 6. Overall ranking based on fifteen parameters analyzed for the species listed below.

Species	Total number of points	Scoring least desirable or next to least desirable of 15 factors	Relative ranking
Primates			
Human			
Homo sapiens	92	13	6
Rodents			
Rat			
Rattus norvegicus	56	4	3
Mouse			
Mus domesticus	59	4	4
Artiodactyla			
Bull			
Bos taurus	65	7	5
Boar			
Sus scrofa	41	1	1
Lagomorpha			
Rabbit			
Oryctolagus cuniculus	51	2	2

Griswold, 1993). The perinatal period, when the size of the Sertoli cell population is established by close control over the timing of mitotic proliferation, is critical for the development of quantitatively normal spermatogenesis in the adult (Orth, 1986). Sertoli cells have differing capacities to support germ cell development and there are wide variations in the numbers of germ cells associated with individual Sertoli cells (Russell & Peterson, 1984) (Table 2). There is a high correlation between the total numbers of Sertoli cells in any testis and the germ cell population that can be supported by them (Orth *et al.*, 1988). Thus animals with more Sertoli cells have more germ cells, and this can be seen in Tables 2 and 5, where the number of Sertoli cells per gram of tissue combined with the number of spermatids per Sertoli cell is positively related to sperm production per gram of testis.

Whereas Sertoli cell numbers are a significant defining parameter for sperm production, it is also important that the Sertoli cell population must not be too large or occupy too much of the seminiferous tubule. The relative volume or volume density of the Sertoli cells within the tubule is inversely related to the efficiency of sperm production (Tables 2 and 5). Russell *et al.* (1990) in a comparative study of twelve mammalian species (including man) showed that there was a strong negative correlation ($r = -0.83$; $p<0.05$) between the volume occupancy of Sertoli cells and sperm production. There are wide species-related variations in this component within the seminiferous tubule compartment (Table 2).

The greatest influence on germ cell production is the capacity for cell division. Most of this is by mitosis among the spermatogonial population. Thus, the number of generations of spermatogonial divisions dictates, in part, the numbers of cells that enter meiosis (Table 3). Spermatogonial production via mitosis is partially offset by apoptotic spermatogonial degeneration (Roosen-Runge, 1973; Blanco-Rodriguez, 1998), possibly mediated by the p53 tumor suppressor protein (Yin *et al.*, 1998[a,b]) and by the genes *Bcl-2 Bax* and *Fas* (Woolveridge *et al.*, 1998, 1999). One possibility - yet to be tested - is that this degeneration may be a homeostatic mechanism to limit germ cells to the number that can be supported by the available Sertoli cells (De Rooij & Janssen, 1987; De Rooij & Lok, 1987).

Meiotic cell loss is also common, especially in humans (Table 3). Missing generations of spermatocytes and spermatids in the seminiferous epithelium caused by lower numbers entering this cohort, plus degeneration, contribute to this low efficiency of human spermatogenesis (Johnson *et al.*, 1992, 2000). Cell kinetics impacted by cell degeneration allows us to express efficiency by a single number: the yield of round spermatids per type A1 spermatogonium (Table 3).

The rate at which cells progress, from spermatogonia to maturing spermatids, is another factor that influences sperm production (Table 4). The overall duration of spermatogenesis is determined by the length of the spermatogenic cycle, under the control of the germ cell genotype (França *et al.*, 1998) (Table 4). Epididymal transit times and extra-gonadal reserves also determine how soon sperm will be available for delivery to the female after formation in the testis (Table 4).

The availability of sperm is one central factor that determines reproductive capacity. This is measured in daily sperm production (DSP) or DSP per unit mass of testis plus epididymal sperm reserves (Table 5).

Finally, the quantity and "quality" of sperm in the ejaculate reflect all that has taken place in the epididymis and the testis. It is difficult and perhaps meaningless for this particular review to attempt to draw up a table comparing semen characteristics between species. There are extremely wide variations in copulatory patterns in mammals (Dewsbury, 1972) and in only a few cases are the numbers of sperm actually delivered to the female tract documented. Even the definition of a morphologically "normal" sperm is difficult, despite common use of the term (Chemes, 2000), as in only a few cases has actual sperm morphology been correlated with successful ovum penetration or transit of reproductive tract barriers (Katz *et al.*, 1990; Liu & Baker, 1994; Krzanowska *et al.*, 1995). Recent work suggests that the process of binding to the zona pellucida selects against aneuploid human sperm, but the mechanism and its association with sperm production mechanisms remains unclear (Van Dyk *et al.*, 2000). Many species (including humans) survive despite high levels of pleiomorphic and supposedly "abnormal" sperm in their semen (Bedford & Hoskins, 1990; Cummins, 1990). Among the Felidae, for example, teratospermia is

widespread and associated with lack of genetic diversity and poor fertility, with associated concerns over their potential to survive in the wild (Pukazhenthi *et al.*, 2001). All we can say at this point is that sperm and semen "quality" is a summation of quantitative and qualitative factors that give fertile males a degree of selective advantage over others (Mann and Lutwak-Mann, 1981; Bedford & Hoskins, 1990; Bedford, 1991[a]). Some of the qualitative problems that affect human fertility in particular appear to be due to deficiencies in Sertoli-germ cell interactions, leading to the premature release of immature cells with a high propensity to free-radical induced dysfunction (Huszar *et al.*, 1998; Huszar & Vigue, 1994).

It is obvious that some of the factors involved in testicular and epididymal function are interrelated in as far as they influence fertility. In other words, the ratings in Tables 1 through 5 are not independent measures, but are factors that combine to influence the final picture. We have attempted to draw a final scoresheet of how humans compare to other heavily studied species in Table 6. This summarizes the ranks set out in Tables 1 to 5 and demonstrates that the human male is worst or next to worst in 13 of the 15 categories.

How Do Humans Compare With Other Mammals?

It is clear from the comparative results summarized above that even normally fertile humans sit at the bottom of the known league table, in terms of most measures of testis efficiency. Johnson (2000) recently expressed the same conclusion, in a paper published after we prepared these tables. He did not speculate on the possible causes, although pointing out, correctly, that the poor performance of the human testis has had little impact on the global population explosion. Even the pattern of human spermatogenesis appears disorganized, with different regions of tubules entering different stages in a mosaic pattern, compared with the regular waves seen in most other species (Setchell, 1982). However, this might reflect a more generalized Primate feature, as "helical" waves are seen in the Marmoset (Millar *et al.*, 2000).

In only two measures are humans not the worst or next to worst among primates. Gorillas resemble humans in that they have relatively small testes per unit body mass and show high levels of sperm pleiomorphism and abnormalities (Martin *et al.*, 1975; Seuanez *et al.*, 1977; Harcourt *et al.*, 1981; Short, 1985). This is a significant observation as it suggests that the relatively poor performance of the human testis is not simply due to recent environmental or societal factors (we return to this topic in the section discussing the genetics of spermatogenesis, below). One possible explanation is that this common feature reflects relatively low intensity of sperm competition between males in both species (Cummins, 1990), but the genetic and evolutionary mechanisms for this are not yet clear. Orang-Utans also have relatively small testes (Dahl *et al.*, 1993) but have very uniform sperm morphology (Martin *et al.*, 1975). Moreover: humans are phylogenetically closer to chimpanzees than gorillas and yet chimpanzee sperm are of excellent quality (Gould & Young, 1996). Chimpanzees also have large testes, consistent with their highly promiscuous mating habits driving high levels of sperm competition (Martin *et al.*, 1975; Harcourt *et al.*, 1981; Harcourt, 1997; Gomendio *et al.*, 1998; Harcourt, 2000). However even this does not guarantee reproductive success within the group. Recent work has suggested that more than 50% of chimpanzee offspring may be fathered by males from outside the immediate troop, and yet the "furtive copulations" that must have led to this have never been observed (Gagneux *et al.*, 1997[a]; Gagneux *et al.*, 1997[b]). This is extraordinary given the high levels of aggression

shown by males to those from other groups (Wrangham, 1997, 1999).

We emphasize that data based purely on testicular parameters are limited and tell us nothing about other aspects of fertility such as mating patterns and the potential for sperm competition (Smith, 1984; Gomendio *et al.*, 1998; Birkhead, 2000). One intriguing point is that the sperm's energy (and ultimately its fertilizing ability) is derived from its mitochondria, which are maternally derived (Cummins, 2000; Moore & Reijo-Pera, 2000). Some reports have already identified differences in sperm motility based on mitochondrial haplotype (Ruiz-Pesini *et al.*, 2000; Wei & Kao, 2000). Moreover at least one point mutation in human mtDNA appears to interfere with successful sperm maturation (Holyoake *et al.*, 1999).

Temperature and the Testis

One suggestion that frequently turns up in the popular literature is that modern human testicular function is adversely affected by temperature, by occupation and specifically by tight clothing. Certainly in a clothed, sitting man scrotal temperature can rise significantly, to a level that might inhibit both testicular and epididymal function (Bedford, 1991[b]) and elevated scrotal temperatures are associated with significantly reduced sperm concentrations in normal men attempting to conceive (Hjollund *et al.*, 2000). Minor elevations of temperature, such as that seen in cases of varicocele, appear to be associated with poor semen quality and with elevated levels of reactive oxygen species and reduced seminal antioxidants (Hendin *et al.*, 1999), but the literature on temperature and human fertility is far from conclusive (Morgentaler *et al.*, 1999). The critical control studies of semen parameters in naked men living in a Rousseau-like "noble savage" environment, are experiments that have never been done and that are probably impossible. Moreover, the poor semen picture seen in Gorillas and other species where there is presumably low intensity of selection on fertility (Cummins, 1983; Cummins, 1990) suggests that such phenomena can exist independently of lifestyle factors. However at least one recent report suggests that elevated heat stress may be associated with poor semen parameters and that cooling the testis may be beneficial (Jung *et al.*, 2001).

Declining Sperm Counts?

There are recent —and controversial—meta-analyses that purport to show declining sperm counts in normal men in industrialized societies (Carlsen *et al.*, 1992,1993; Ulstein, 1996; Van Waeleghem *et al.*, 1996; Irvine, 1997; Swan *et al.*, 1997; Giwercman & Bonde, 1998; Ulstein *et al.*, 1999; Swan, *et al.*, 2000). Variations in the rate of decline have been found between fertile donors and infertile patients (Gandini *et al.*, 2000). However there is no such decline in sperm counts from farm animals, so if environmental factors are at work they must have an especially heavy impact on humans (Setchell, 1997). The estimates based on crude sperm counts have been criticized as methodologically flawed (Lerchl & Nieschlag, 1996; Handelsman, 2000). However, the hypothesis of a general decline in male fertility is supported by more solid evidence of increasing levels of reproductive developmental anomalies and testicular cancer (Moller, 1998). There is also evidence of reduced male: female sex ratios (Davis *et al.*, 1998) and declining levels of dizygotic twinning (James, 1998) as indices of general fertility. While environmental factors such as xenoestrogens and androgen mimics and antagonists are generally suspected to play a role in declining human male fertility (Turner & Sharpe, 1997; Sonnenschein & Soto, 1998) the exact causes are likely to be complex and multifactorial. In addition, changing reproductive decision-making means that

fertility is increasingly likely to be compromised by age, as there is a widespread human trend to delay parenting (Tarín *et al.*, 1998).

Human Lifespan and Reduced Fecundity

A low level of investment in human reproduction may have evolved as an inevitable trade-off in the evolution of longevity and a complex culture, according to Kirkwood's "disposable soma" hypothesis (Kirkwood & Rose, 1991; Kirkwood, 1997). This postulates that as the effects of natural selection are weaker at later ages once genes have been transmitted, the costs of maintaining the somatic tissues for an extended lifespan are at the expense of investment in reproductive effort. For women, this is partly supported by longitudinal genealogical studies showing that overall reproductive success is lower in very long-lived women (Westendorp & Kirkwood, 1998; Korpelainen, 2000) and that women who give birth late in life have greater life expectancy (Perls *et al.*, 1997). Late-reproducing women also have less fertile sons (teVelde, personal communication), indicating that the two traits may be linked between the generations. By contrast, another study found that the interaction between reproductive success and longevity is more a function of economic factors and social rank (Lycett *et al.*, 2000). Gerontologists generally agree with William's hypothesis of antagonistic pleiotropy, which posits a mix of effects, with some genes conveying fitness benefits in early life but deleterious effects in old age. However, the specific trade–off postulated by Kirkwood does not appear to hold up, at least for those human and primate models that have been evaluated rigorously (Le Bourg, 2001).

The possibility therefore that genes "for" reduced or delayed fecundity may have co-evolved with those predisposing for longevity — as opposed to the simple loss or degradation of normal genes — remains an open question. Intriguingly, certain mitochondrial haplotypes are also associated with longevity through their control of overall bioenergetics and aging (De Benedictis *et al.*, 1999, 2000; Korpelainen, 1999). How these complex interactions affect males is still far from clear. Increased paternal age is linked to reduced fecundity (Ford *et al.*, 2000) and to increased germline mutations (Sawyer & Aitken, 2000). Cumulative male reproductive success is associated with increased longevity (Korpelainen, 2000) meaning that low individual fecundity can be compensated for by living longer. Reduced human fertility may thus have emerged as a side-effect of lifespan evolution through relaxed selection pressures leading to slightly deleterious mutations accumulating in the gene pool.

Among primates, humans have an unusual reproductive pattern with cryptic oestrus (Sillén-Tulberg & Møller, 1993), moderate sexual dimorphism, and great elaboration of the penis and breasts as secondary sexual signals (Lerchl, 1997). Along with only one other mammal - a species of toothed whale (Peccei, 1995) - humans have a genetically programmed period of female infertility at late maturity. The menopause possibly evolved to reinforce cultural transmission between generations - the "grandmother hypothesis"(Hawkes *et al.*, 1998, 2000). While our relatively small testes suggests that sperm output *per se* is relatively unimportant, men are about 10-15% taller than women and this sexual dimorphism suggests that we evolved a pattern of effective moderate polygyny through "serial monogamy" (Short, 1997). Some form of polygyny – serial or simultaneous – is practiced in over 80% of human cultures (Short, 1994; Shoumatoff, 1995; Lerchl, 1997). Most women in pre-contraceptive gathering-hunting societies appear to have assured that births were spaced about every 3-4 years. This was achieved by a

combination of lactational amenorrhoea, induced abortion, infanticide or avoidance of intercourse (Daly & Wilson, 1983; Hrdy, 1999). Indeed in ecological terms humans are "K-selected" – large, slow-growing, long-lived animals with relatively high rates of reproductive wastage and massive investment in a few offspring at a time (Short, 1985). Infertility may be an extension or exaggeration of this trend, in which case it may be futile seeking "adaptive" explanations for its cause. It would be valuable to examine long-term male reproductive success in other very long-lived "K"-selected mammals such as elephants and whales. Other aspects of human (and some primates') reproduction, such as cryptic oestrus (Sillén-Tulberg & Møller, 1993) and menstruation (Finn, 1998) likewise may be non-adaptive features that have emerged as side effects of selective pressures on other behavioral and physiological traits. It is known, for example, that slightly deleterious mutations can accumulate in response to relaxed selection pressures in Drosophila (Lynch *et al.*, 1999). Reduced fecundity may therefore have little or no direct adaptive significance.

There seems little doubt that the emerging information on the human genome will also shed light on the genetic control of spermatogenesis (Escalier, 2001; Olesen *et al.*, 2001). Many genes affect testicular function, including those controlling the hypothalamo-pituitary axis, the development of the urogenital system and hormone receptors and target organs (Nieschlag, 1997; Tut *et al.*, 1997; Ghadessy *et al.*, 1999). Sperm and seminal plasma together have more than 1700 expressed proteins (Naaby-Hansen *et al.*, 1997), and the number of genes involved in spermatogenesis may be as high as 4000 (Hackstein *et al.*, 2000); or 3-11% of the estimated 35-40,000 in the human genome (Aparicio, 2000; The Genome Sequencing Consortium, 2001; Venter *et al.*, 2001). Most genetic causes of idiopathic infertility are probably due to autosomal recessive mutations (Lilford *et al.*, 1994), but most of the critical genes affecting spermatogenesis have become concentrated on the sex chromosomes: both the Y (Charlesworth & Charlesworth, 2000; Graves, 1998; Graves, 2000; Jobling & Tyler-Smith, 2000; Quintana-Murci *et al.*, 2001) and, perhaps surprisingly, the X. The rapid expansion of information on the human genome (Venter *et al.*, 2001) will undoubtedly improve our understanding of the complex evolutionary forces underlying the uniquely poor reproductive performance of the human male.

The Y Chromosome

There are at least three regions in the euchromatic section of the Y chromosome (Yq11) containing genes affecting spermatogenesis (Vogt, 1997; Vogt, 1998; Quintana-Murci *et al.*, 2001). These have been designated AZFa, AZFb and AZFc (Azoospermia Factor), and deletions in each gives rise to different testicular pathology. Saxena *et al.* (2000) found four full-length DAZ genes on the human Y chromosome, in two clusters each comprising an inverted pair of DAZ genes: with multiple tandem copies of a 2.4-kb repeat unit encoding for 24 amino acids. An autosomal homologue to DAZ, DAZL, is also present (it is the only copy in mice). The DAZ/DAZL genes encode for cytoplasmic RNA-binding proteins found in the nuclei of foetal gonocytes and spermatogonia that relocate to the cytoplasm in post-meiotic cells (Reijo *et al.*, 2000). The genes (and possibly their functions) are strongly conserved among the primates (Grossmann *et al.*, 2000). These deletions and reduplications of sequences result from internal recombination between misaligned sister chromatids (Vogt, 1990): some of these apparently result from crossovers between repeat human endogenous retroviral (HERV) sequences (Blanco *et al.*, 2000; Kamp *et al.*, 2000; Sun *et al.*, 2000). Thus, male infertility may be added to a growing list of diseases caused by HERV-related microdeletions and duplications (Lupski, 1998). This

is not surprising. About half of the human genome consists of transposable elements and interspersed repeats (Smit, 1999; The Genome Sequencing Consortium, 2001) and HERV sequences make up about 7% (Bock & Stoye, 2000). When one couples this noisy genetic scenario with longevity, high mutation rates and relaxed selection pressures on fecundity for humans, it is hardly necessary to postulate a single genetic "cause" for impaired spermatogenesis.

Control of spermatogenesis is clearly based on a genetic network or networks acting in a cascade, with mutations causing multiple problems ranging from total meiotic failure to faulty spermiogenesis (Krausz & McElreavey, 1999; Venables & Cooke, 2000). The notion that any biological trait is absolutely controlled by a single gene is largely defunct: the human genome contains only about 35 – 40,000 genes (The Genome Sequencing Consortium, 2001; Venter *et al.*, 2001)and studies in gene knockout mice show that many single gene deletions that affect spermatogenesis when on both chromosomes can be compensated for when only present on one (Escalier, 2001). For the testis, some genes (e.g. RBM and DAZ) are now found on the Y chromosome in multiple copies and are only expressed in the testis, however they have evolved from autosomal homologues (Saxena *et al.*, 1996; Chai *et al.*, 1997; Lahn & Page, 1997). There are also "housekeeping" Y genes with homologues on the X chromosome, which are expressed in multiple tissues. Many genes affecting fertility in both males and females have been identified by induction of chromosomal changes in mice and rats and this list is likely to grow rapidly (Okabe *et al.*, 1998; Russell & Griswold, 1999; Venables & Cooke, 2000). These findings are consistent with the general view of the evolution of the sex–determining chromosomes, suggesting a progressive loss or degradation of genes not essential for fertility (Charlesworth & Charlesworth, 2000; Graves, 2000).

The X Chromosome and Spermatogenesis

While the analysis above concentrated on the Y chromosome, surprisingly a majority of genes controlling early (diploid) spermatogenesis have become concentrated on the X chromosome: of 25 genes specific to spermatogonia, no fewer than 10 are X-linked while only three are Y-linked (Wang *et al.*, 2001). This may appear paradoxical, however Wang *et al.* put forward two hypotheses: one based on meiotic drive and one based on sexual antagonism. Meiotic drive refers to the tendency for genes controlling reproductive fitness to become concentrated on the sex chromosomes at the expense of the autosomes (Frank, 1991). This divergence of segregation also helps explain Haldane's rule that, when species hybridize, the heterogametic sex is much more likely to be sterile, unviable or absent (Haldane, 1922). The spermatogonial X-linked genes may therefore act as drivers of X transmission or Y suppression resulting in skewed transmission to the gametes and thus increased transmission to the offspring. As the Y chromosome is inherently unstable (discussed above), it may also be that the genes are "protected" by being localized on the relatively safer X. The intriguing question is whether such meiotic drive might drive male infertility and thus enhance the transmission of X chromosomes? There are actually several known examples of genes affecting sex-biased loss of fertility and viability on the X chromosome (Frank, 1991). The second hypothesis is based on the likelihood that sexually antagonistic recessive genes, that enhance fitness in one sex but reduce it in the other (for example antlers in deer) will become fixed on the X chromosome because the benefits are only expressed in the XY condition (male) but suppressed in the XX (Rice, 1984; Hurst, 2001).

Other Genetic Factors Affecting Spermatogenesis

About 4-5% of otherwise healthy men suffer from unexplained infertility (Baker *et al.*, 1986; World Health Organization, 1987; Bhasin *et al.*, 1994). The Online Mendelian Inheritance in Man database (http://www.ncbi.nlm.nih.gov/Omim/) lists 50 single genes involved in infertility in human males. The majority of genetic infertility in the general population is probably due to recessive autosomal genes as the X- and Y-linked disorders that cause sterility are self-limiting – or at least were until the advent of techniques such as ICSI allowed parenting for very severely affected men (Cram *et al.*, 2000; Hackstein *et al.*, 2000). The importance of autosomal recessives is underlined by the familial nature of idiopathic infertility (Lilford *et al.*, 1994) and strong links with elevated levels of consanguineous marriage (Zlotogora, 1997). There are intriguing examples of degenerating genes affecting fertility that are not on the Y chromosome. Thus, the haploid-expressed gene for the sperm-specific endozepine-like peptide (ELP) encodes for a protein with marked homology to the acyl—CoA binding protein that mediates intracellular fatty acid transport to the mitochondria for b-oxidation. A comparative evaluation of this gene between old and new world primates has revealed a gradual process of down-regulation and mutation, suggesting that the primates as a group have been able to tolerate the gradual increase in genes disposing to infertility (Ivell *et al.*, 2000). There are five known members of the mammalian fertilin/ADAM/MDC family of sperm surface proteins, which are believed to be important for sperm-egg interaction. However, only two of these remain functional in humans (Hall & Frayne, 1999). Other gene systems known to be defective in infertile men include those of the cAMP responsive element modulator (CREM) activators (Behr & Weinbauer, 2000). The list is likely to grow as the Human Genome Project reaches fruition. Research based on gene knockout and transgenic mice has already mapped over thirty genes acting to interrupt or impair the spermatogenic cascade (Venables & Cooke, 2000). One example is the gene for protein C inhibitor, where the knockout mice show malformed sperm and abnormal spermatogenesis due to Sertoli cell dysfunction (Uhrin *et al.*, 2000), similar to that seen clinically in infertile men (He *et al.*, 1999). Alternative approaches would be to use spermatogonial stem cell transplantation into animals with known or modified genetic backgrounds (Johnston *et al.*, 2000).

Conclusions

We demonstrate that human males sit at the bottom of the league table in almost every measure of testicular function and efficiency. This should be regarded with caution, as the human data are based almost exclusively on modern individuals whereas those for animals are based on species that in most cases have been selected for fertility. Infertility in present populations may be an outlier effect of the low fecundity that in general appears to be endemic to human (and primate) reproduction. Other aspects of human reproductive biology, such as the menopause and menstruation, are considered by some to be non-adaptive features (Finn, 1998; Hawkes *et al.*, 1998) that have been retained because, on balance, they do not reduce fitness. The relaxed natural selection processes that have led to modern humans may have resulted in a trade-off between investment in fecundity and in factors associated with cultural transmission such as symbolic language and longevity.

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