Relationship between urinary estrogen levels before conception and sex ratio at birth in a primate, the gray mouse lemur

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BACKGROUND: In primates, including humans, bias of sex ratio at birth has been reported to depend on maternal condition at conception. In a Malagasy primate, the gray mouse lemur, male-biased sex ratio at birth occurred in captive parous females. The male bias was previously demonstrated to be pre-conceptual and independent of the female’s nutritional state. It was hypothesized to be related to changes in hormonal state at ovulation.

METHODS and RESULTS: The relationship between urinary estradiol ($E_2$) levels during the follicular phase until estrus and litter production (number and sex of newborns) was assessed in 91 females mated under controlled conditions. Changes in $E_2$ levels prior to ovulation followed the typical primate pattern characterized by a gradual rise during the 10 days preceding the sharp increase at estrus. A clear decline in $E_2$ levels occurred with ageing. Direction of the sex ratio bias was unrelated to $E_2$ levels at ovulation time but was significantly dependent on $E_2$ levels during the follicular phase. Reduced $E_2$ levels prior to estrus led to male-biased litters.

CONCLUSION: This study suggests that hormonal stimulation during the follicular phase plays a role in shifting sex ratio at conception through changes in the local environment of the ova. This hypothesis deserves testing by assessing estrogen levels throughout the follicular phase in other primate species including humans.

Key words: body condition/estrus cycle/maternal hormones/sex ratios/sperm selection

Introduction
In polygynous mammals including primates, maternal ability to vary the sex of her offspring is considered as adaptive. Several examples of a shift in the sex ratio at birth have been reported, but controversial explanations were provided for the direction or the environmental determinism of sex ratio bias (Clutton-Brock and Iason, 1986; Gomendio et al., 1990; Hiraiwa-Hasegawa, 1993). In most species, maternal condition at the time of conception has been considered as the proximate mechanism for biased sex ratio at birth. However, maternal condition may be affected by various intricate environmental factors. The question of which mechanisms might be responsible for biasing sex ratios at birth remains under debate (Sieff, 1990; James, 1995, 2004; Krackow, 1995; Mendl et al., 1998; Packer et al., 2000).

Except species for which biased sex ratios at birth have been demonstrated to rely on post-conceptual manipulation (Pratt and Lisk, 1989; Krackow, 1995; Kruuk et al., 1999), several mechanisms for a pre-conceptual bias have been proposed. The timing of copulation relative to the moment of ovulation might be a factor since Y-bearing sperm move faster and could reach the ovum earlier than X-bearing sperm (James, 1985a; Hedrick and McClintock, 1990; Hornig and McClintock, 1996). The influence of gonadotrophic and of sex hormone levels at conception has also received great attention (James, 1996, 2004). In females of below average condition, stress effects and their resulting decrease in sexual hormones have been evoked to explain increasing probability of female offspring. Likewise, the reduced ratio of male to female births in industrial countries might be interpreted as a signal of decreasing health condition in women linked to increasing exposure to hazardous environmental conditions (James, 1992; Davis et al., 1998; Parazzini et al., 1998; Astolfi and Zonta, 1999). However, in women, high gonadotrophin levels used for induction of ovulation have been associated with an excess of female births (James, 1985b, 1990, 1992). Thus, the effect of hormonal levels, which in turn are under the influence of stress hormones or of body condition, appears to be extremely complex and the physiological processes whereby fluctuations of hormones may underlie variation in sex ratio at conception remain unclear.

The low fecundity of most primates renders the mechanism of sex ratio bias difficult to study. In contrast, the gray mouse lemur, a small Malagasy prosimian primate...
(Microcebus murinus) is a convenient species to study bias of sex ratio at birth. Reproduction in this species is highly seasonal. Females exhibit marked estrous synchrony at the beginning of each reproductive season and they give birth to one to three babies of 6–7 g, after a gestation period of ~60 days. As in some other captive prosimians (Watson et al., 1996), female mouse lemurs demonstrated a significant male-biased sex ratio at birth, which is independent of the female’s nutritional state or litter size (Perret, 1990). Females over-produced sons when they were maintained in large groups prior to mating, whereas isolated females presented an inverse tendency (Perret, 1990). Sex ratio bias towards one sex or the other was demonstrated to be pre-conceptual and linked to social interactions between females during the follicular phase until estrus (Perret, 1995, 1996). A deficit in sexual hormones at ovulation has been hypothesized for a pre-conceptual bias. High hormonal levels would lead to a bias towards females whereas a reduction, induced by social cues between females, would be responsible for sex ratio bias towards males.

The aim of this study was to examine the relationship between estrogen levels during the follicular phase until estrus and litter production in terms of number and sex of newborns produced in captive female mouse lemurs mated in controlled conditions.

Materials and methods

Animals

Ninety-one adult female gray mouse lemurs (Microcebus murinus) were used in this study. All animals were born in the laboratory breeding colony (Brunoy, MNHN, France, European Institutions Agreement no. 962773) from a stock originally caught in southern Madagascar 35 years ago. They were kept in controlled conditions with constant ambient temperature (24–26°C), constant relative humidity (55%) and food available ad libitum. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

Biological rhythms of mouse lemurs are dependent on photoperiod. Exposure to long days (>12 h light per day) entrains seasonal activation of reproductive function associated with increased behavioural and physiological activity. In contrast, exposure to short days (<12 h light per day) leads to pronounced fattening, reduced activity, torpor and complete sexual rest in both sexes (Perret and Aujard, 2001). In the breeding colony, to ensure highly synchronized changes in biological rhythms within individuals, animals were exposed to an artificial photoperiodic regimen consisting of a 3 month period of Malagasy winter-like short day-length and a 5 month period of Malagasy summer-like long day-length. Age-specific survival curve of female mouse lemurs has been previously established in the large colony of Brunoy (Perret, 1997) and aged animals have been defined as those that are older than the 50% survival age (Burek, 1978). In female mouse lemurs whose potential longevity reaches 12 years, the 50% survival occurred at ~5 years, i.e. after the 5th breeding season (Perret, 1997). Consequently, studied females were separated into three categories of age according to the number of seasonal cycles they had experienced: young adults (1st breeding season); adults (from the 2nd to the 5th breeding season); and aged females from the 6th to the 11th breeding seasons. All the young females were nulliparous since experiencing the first breeding season of their life. Adult and aged females were parous except for five females in which their first pregnancy occurred during the 2nd or 3rd breeding season.

Induction of estrus

In artificial photoperiodic conditions, females entered estrus almost synchronously within 30–35 days after the stimulation by long day-length. Females were kept in single sex groups of 2 or 3 individuals. For each female, the follicular phase can be monitored by the striking morphological changes of the vulva preceding estrus (pinkening, progressive enlargement, large swelling and perforation). To ensure insemination, females were placed in contact with breeding males 3 days before the perforation of the vulva. Females mated 1–2 days after perforation of the vulva, i.e. before ovulation which normally occurs spontaneously on the 3rd day (Perret, 1986). Body mass at estrus was recorded and insemination was verified by vaginal smears. All inseminated females were isolated and a diagnosis of pregnancy was performed by abdominal palpation ~1 month after mating. Gestation lasts ~2 months. Spontaneous abortions were observed 1–2 weeks before the expected time of delivery. Abortion can be detected by a large bloody vaginal opening, with or without visible fetal remains, and is associated with a loss of body mass and a return to estrus a few days later. At birth, litter size (from one to three young) and litter composition were recorded. According to the sex of each newborn produced (M = male and F = female, visually distinct from birth), the type of the litter was defined as well balanced for MF litters, as male-biased for M, MM, MF and MMM litters and female-biased for F, FF, FFM and FFF litters.

Collection of urine

Two weeks after the exposure to long daylength, urine was collected every day on each female from the beginning of the follicular phase until the 4th day following perforation of the vulva. Volumes of urine from 1 to 3 ml were spontaneously voided within 2 min after females were removed from their nest. Sampling was made at a fixed time during the diurnal resting phase. Urine samples were stored at ~20°C until assayed.

17β-Estradiol (E2) measurements

Urinary E2 concentrations were measured on 25 μl of urine in duplicate using an enzyme-linked immunosorbent assay (IBL, Germany). Percentages of cross-reactivity were: E2 100%, estrone 2.1%, estriol 1.5%, other steroids tested <0.1%. The mean intra- and inter-assay coefficients of variation was 3.3 and 4.9% respectively. The minimum detectable level in urine was 10 pg. To take into account individual variations in urine concentration, the creatinin (Cr) content was measured in each sample using a colorimetric test (Sigma Diagnostics, USA). Values of urinary E2 were expressed in pg/mg Cr.

Statistical analysis

Days are referred from the day of vaginal opening. All values are mean ± SEM. Statistical analysis included the χ²-test and G-test for distribution, multiway analysis of variance or analysis of covariance with body mass, litter size or age as co-factors and non-parametric tests (Kruskal–Wallis or Friedman) for mean differences according to the normality of the data, linear correlations between parameters and the paired t-test for deviation in sex ratios. For statistical analysis of sex ratio at birth, we analysed the proportion of litters in which infants of one sex predominated (male-biased or female-biased, irrespective of the litter size) compared with well-balanced litters.
Results

Reproductive parameters of the female population studied

Before testing the possible relationship between E2 levels during follicular phase until estrus and sex ratio bias at birth, the homogeneity of the different parameters (body mass, abortion, pregnancy, litter size, litter type) within the 91 females was tested according to age categories (Table I). Female mouse lemurs reproduce throughout life since successful pregnancies were still observed at an advanced age (until the 11th breeding season).

Females entering their first breeding season exhibited a significantly lower body mass (mean = 75.1 ± 2.4 g, n = 19; Table I) compared to all other females (mean = 96.5 ± 2.7 g, n = 72, F = 21.3, df 1/89, P < 0.001) for which no correlation existed between age and body mass (r = 0.01, n = 72, not significant). Spontaneous abortions were observed 51 ± 1 days on average after copulation (n = 24), independent of body mass (r = 0.262, n = 24, not significant). Although a higher proportion of abortions occurred in aged females, no significant difference was observed according to female age categories (G = 2.2, df2, not significant).

Profile of urinary E2 during the peri-estrus period

Urinary E2 concentrations were monitored during the 10 days before the perforation of the vulva (corresponding to the follicular phase visually distinct by a progressive enlargement and pinkening of the vulva) and during 4 days following the vulva opening. The turgescence of the vulva was at its maximum 1 day before the perforation of the vulva (corresponding to the follicular phase; Figure 1). Changes in urinary E2 levels were characterized by a progressive and significant linear increase (~30 pg/mg Cr per day, r = 0.975, df 9, P < 0.001), with values increasing greatly a few days before the perforation of the vulva. Maximal values were observed during the day of vaginal perforation (mean values: 367 ± 18 pg/mg Cr, n = 91). Thereafter, urinary E2 significantly dropped to reach low values 2–3 days after the beginning of vaginal estrus.

Relationship between urinary E2 and age

Significant differences were observed in both profiles and quantity of urinary E2 according to age. Levels of urinary E2 recorded the 1st day of estrus significantly decreased with increasing age (r = 0.716, n = 91, P < 0.001; Figure 2), with significant differences between age categories (F = 40.2, df 2/88, P < 0.001; Table I). Moreover, profiles of urinary E2 during the peri-estrus period significantly differed according to age categories (Figure 3). During the 5 days preceding estrus (late follicular phase), urinary E2 levels in aged females were significantly lower than those of other females (F = 13.3, df 2/88, P < 0.001; Table I). Lastly, within each age category, no significant correlation existed between female body mass and E2 levels at estrus (r = –0.386, n = 19; r = –0.08, n = 50; and r = 0.02, n = 22; for young, adult and aged females respectively, not significant) or E2 levels during the follicular phase (r = –0.07, n = 19; r = –0.04, n = 50; and r = 0.197, n = 22; for young, adult and aged females respectively, not significant).

Relationship between urinary E2 and sex ratio at birth

From the 67 successful pregnancies, the relationship between urinary E2 and litter production was examined. Overall, female mouse lemurs produced preferentially litters of twins (39/67, 58.2%). The distribution of litter size was homogeneous within the different age categories (G = 7.5, df 4, not significant) although young females tended to produce preferentially litters of twins (12/16, 75% versus 27/51, 52.9% in other females). Mean litter size was not significantly different between age categories (F = 0.14 df 2/63, not significant; Table I) and was independent of body mass.

Table I. Body mass and reproductive parameters of females according to age categories

<table>
<thead>
<tr>
<th>Age category</th>
<th>Abortion/successful pregnancies</th>
<th>Body mass (g)</th>
<th>E2 at estrus (pg/mg Cr)</th>
<th>E2 during the late follicular phase (pg/mg Cr)</th>
<th>E2 at estrus (pg/mg Cr)</th>
<th>E2 during the late follicular phase (pg/mg Cr)</th>
<th>Mean litter size (total no. of newborn)</th>
<th>Sex ratio (M/F) (% males)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (n = 19)</td>
<td>3/16</td>
<td>75.1 ± 2.4</td>
<td>517 ± 28</td>
<td>264 ± 21</td>
<td>1.9 ± 0.1 (30)</td>
<td>46.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults (n = 50)</td>
<td>13/37</td>
<td>94.7 ± 4</td>
<td>396 ± 25</td>
<td>271 ± 19</td>
<td>2.1 ± 0.1 (79)</td>
<td>65.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aged (n = 22)</td>
<td>8/14</td>
<td>96.5 ± 4</td>
<td>184 ± 14</td>
<td>125 ± 14</td>
<td>2.1 ± 0.2 (29)</td>
<td>62.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
E2 = estradiol; Cr = creatinin.

Figure 1. Profiles of urinary estradiol (E2) levels (mean ± SEM) during the peri-estrus period according to the result of impregnation: abortion or successful pregnancy.
\( F = 2.6, \) df 1/63, not significant), demonstrating a lack of sex-selective abortion.

The overall sex ratio (% males) of 138 newborn from 67 litters produced differed significantly from the 50% equality sex ratio expectation (84 males/54 females, sex ratio 60.8%, \( \chi^2 = 6.5, \) df 1, \( P < 0.02 \)). The male-biased sex ratio at birth was achieved by a significantly higher proportion of litters in which infant males predominated. Indeed, from the 67 litters produced, 35 were male-biased, 15 were female-biased and 17 were equilibrated, a distribution that significantly differed from the expected binomial distribution (\( \chi^2 = 20.3, \) df 2, \( P < 0.001 \)). Litter composition was found to be independent of the size of the litter (\( G = 0.7, \) df 2, not significant) and was not related to the body mass of the female (\( F = 0.4, \) df 2/64, not significant). The distribution of litter composition was not significantly related to female age category (\( G = 3.0, \) df 4, not significant). However, young females did not bias the sex ratio at birth towards males (sex ratio = 46.7% males, from 30 newborns) whereas all other females demonstrated a significant bias of sex ratio at birth towards males (sex ratio = 64.8% males, from 108 babies, \( \chi^2 = 21.3, \) df 1, \( P < 0.001 \); Table I).

During the follicular phase (from −10 to −1 day before estrus), urinary E2 levels were not significantly different according to the litter size produced (\( KW = 0.2–4.9, \) \( P = 0.1–0.8 \)). However, females that produced litters of three newborns exhibited significantly lower levels of urinary E2 the day of estrus (\( F = 4.9, \) df 2/64, \( P < 0.01 \)), independent of body mass (\( F = 2.3, \) df 1/64, not significant).

In female mouse lemurs, profiles of urinary E2 differed significantly according to the composition of the litter from well-balanced litters (MF) to litters in which infants of one sex predominated (male- or female-biased; Figure 4). Significant reduction in urinary E2 levels appeared during the follicular phase in females that produced male-biased litters compared to levels recorded in females that produced well-balanced or female-biased litters (\( KW = 9.6–16.6 \) with \( P < 0.01 \) to \( P < 0.001 \)). During the late follicular phase, values of urinary E2 differed significantly according to the sex ratio of the litter produced (\( F = 13.5, \) df 2/63, \( P < 0.001 \); Table II), a difference that was not related to body mass (\( F = 0.5, \) df 1/63, not significant), nor to the size of the litter (\( F = 3.4, \) df 1/64, not significant). By contrast, E2 levels at estrus did not vary according to the composition of the litter (\( F = 0.02, \) Table II).

![Figure 2.](image2.png)

**Figure 2.** Age-related changes in urinary estradiol (E2) concentrations at estrus. Age is expressed as the number of breeding seasons experienced by females.

![Figure 3.](image3.png)

**Figure 3.** Profiles of urinary estradiol (E2) levels (mean ± SEM) during the peri-estrus and estrus periods according to female age categories. Significant difference between aged females compared to other females: *\( P < 0.01 \) to **\( P < 0.001 \).

![Figure 4.](image4.png)

**Figure 4.** Profiles of urinary estradiol (E2) levels (mean ± SEM) during the peri-estrus and estrus periods according to the composition of litters from well-balanced litter (one male/one female) to litters in which one sex predominated (male- or female-biased litters). Significant difference between females that produced male-biased litters compared to other females: *\( P < 0.01 \) to **\( P < 0.001 \).

<table>
<thead>
<tr>
<th>Litter composition</th>
<th>Body mass (g)</th>
<th>E2 during the late follicular phase (pg/mg Cr)</th>
<th>E2 at estrus (pg/mg Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female-biased, ( n = 15 ) (F, FF, FFF, FFM)</td>
<td>87.3 ± 4</td>
<td>338 ± 35</td>
<td>399 ± 44</td>
</tr>
<tr>
<td>Well-balanced, ( n = 17 ) (MF)</td>
<td>89.8 ± 4</td>
<td>281 ± 23</td>
<td>378 ± 23</td>
</tr>
<tr>
<td>Male-biased, ( n = 35 ) (M, MM, MMM, MMF)</td>
<td>92.5 ± 3.3</td>
<td>181.5 ± 15</td>
<td>369 ± 30</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Cr = creatinin.
df 2/62, not significant; Table II), suggesting that the sex-biased sperm selection mostly depended on hormonal stimulation during the late follicular phase.

Discussion

Female mouse lemurs reproduce seasonally throughout their life with a high fecundity rate in terms of litter production. A significant male-biased sex ratio at birth occurred in captive parous females. In this primate, sex ratio bias was dependent neither on the mother’s body condition nor on the litter size and was achieved through an excess of males in litters. Male-biased sex ratio at birth was unrelated to E₂ levels at ovulation time but was significantly associated with reduced E₂ levels during the late follicular phase. This suggests a sex-selective sperm selection at conception time, dependent on a hormonal mechanism occurring before estrus.

Profile of urinary E₂ during the estrous cycle

In female mouse lemurs, changes in estrogen levels prior to ovulation follow the typical primate pattern. Urinary E₂ levels demonstrated a gradual rise during the 10 days preceding the sharp increase at estrus, reaching on average 370 pg/mg Cr. As in other primates exhibiting a prominent sex-skin swelling (Thomson et al., 1992; Heistermann et al., 1995; Aujard et al., 1998), the striking morphological changes of the vulva in mouse lemurs were correlated to changes in urinary estrogens, indicating that swelling is likely to be estrogen dependent. The comparison of ovulatory peak steroid levels with other primates remains difficult because the assessment of cyclic profiles in estrogen excretion relies on different measures (mostly estrone conjugates) from urine, faeces or blood, depending on the species studied. However, E₂ levels in plasma have been evaluated to ~200–400 pg/ml in South American primates (Ziegler et al., 1993), in macaques (Walker et al., 1984; Frazer and Sandow, 1985; Thomson et al., 1992), in higher primates (Nadler et al., 1979; Nadler, 1984) and 100–200 mg/ml in women (Vom Saal et al., 1994), values comparing well with those found in female mouse lemurs.

With ageing, profile of urinary E₂ levels flattened during the follicular phase and at estrus. This agrees with the obvious reduction of estrogen levels described in primate species with ageing (Vom Saal et al., 1994; Wasser et al., 1998) accounting for reduction in fertility. However, in female mouse lemurs, the decrease in estrogen levels has no bearing on the conception rate and the number of ova shed seems to remain relatively constant throughout the reproductive life. In this primate, ageing is not associated with a fertility loss or menopause state and is even associated with a displacement towards large litters compared to younger females. The seasonal reproductive pattern of mouse lemur female includes a complete ovarian atrophy during the winter season and the reactivation of follicular growth occurs in spring from primordial follicles. This particular feature could explain the absence of oocyte loss during ageing. The fertility decline in old females in terms of number of pregnancies is mainly due to a decrease in sexual behaviours (attractiveness and receptivity) for successful mating.

Relationship between E₂ levels and sex ratio at birth

A significant relationship was demonstrated between estrogen levels during the 5 days prior to estrus and the composition of the litter produced: estrogens were significantly reduced in females that produced male-biased litters, independently of the size of the litter. How might estrogen levels during late follicular phase play a role in shifting sex ratio at conception? During late follicular phase, the greatest quantities of estrogen come from large antral follicles and the reduced estrogen production reflects both inappropriate secretion of gonadotrophins and low development of granulosa cells (Gore-Langton and Armstrong, 1994). With regard to mammalian fertilization of the ovum, three levels could be implicated in sex sperm selection: the zona pellucida, the cumulus oophorus and the follicular fluid. These structures are implicated in capacitation, filtration and attraction of sperm (Yanagimachi, 1994) and need specific hormonal requirements at precise periods during the follicle development. The cumulus oophorus and the follicular fluid appear at a later stage and depend on gonadotrophin secretion/estrogen levels to function appropriately. Lowered stimulation by estrogen at a later stage of the follicle development would modify the composition of the follicular fluid and the structure of the cumulus oophorus. The fluid of the antral follicle is known to contain, in addition to estrogens, varied substances some of which play a definite role in sperm attraction (Ralt et al., 1991; Gore-Langton and Armstrong, 1994). Likewise, the cumulus oophorus of which the primary function is a sperm-sequestering device expanded according to gonadotrophin stimulation (Bedford and Kim, 1993; Krackow, 1995). A diminution of sperm attraction and a low development of cumulus oophorus through reduced stimulation by estrogen would favour Y-biased sperm that move faster, thus leading to male-biased sex ratio at birth. In several mammals including non-human primates, the male:female ratio at birth increases with ageing (Rawlings and Kessler, 1986; Huck et al., 1988; Mace, 1990; Cassinello and Montserrat, 1996) and could be also dependent on changes in hormonal environment at conception.

Lastly, the timing of fertilization might determine sex ratio bias as the result of behavioural differences between the populations of X- or Y-bearing sperm with, however, a controversial direction of the shift towards one sex or the other according to mating before or after ovulation (James, 1985a; Hedrick and McClintock, 1990; Horning and McClintock, 1996; Jimenez et al., 2003). In our experimental conditions to ensure insemination, mouse lemur females mated at the same time relative to ovulation, i.e. always before ovulation (Andrés et al., 2003), and sex-biased sperm selection according to mating time is unlikely to explain offspring sex ratio bias. Likewise, although paternal hormonal condition has been suspected to partially control sex ratios at conception (James, 2004), sexual competition in mouse lemurs leads to the emergence of a dominant male that exhibits high testosterone levels and has priority access to receptive females (Perret, 1995).
Environmental factors and sex ratio bias

In mouse lemurs, which are probably long-photoperiod seasonal breeders, rapid changes in gonadotrophins and prolactin secretions occur in spring, accounting for the reactivation of ovarian function from primordial follicles to ovulatory state in a few weeks. In captivity, sex ratio bias towards males in grouped females during the follicular phase has been demonstrated to be linked to olfactory cues (Perret, 1996). Indeed, shifts in sex ratio at birth towards female or male newborn can be obtained in females isolated before conception by exposing them or not to urinary odours from grouped females. Chemical cues would lead to inappropriate secretion of gonadotrophin and/or prolactin hormones, independent of direct social contacts, as exemplified in rodents or primates for intrasexual inhibition of ovulation in females (Abbott, 1993). However, other sexual hormones should be involved, such as female testosterone levels around the time of conception (James, 2004). In female mouse lemurs, plasma testosterone levels at estrus time were very low, <0.5 ng/ml, and presently no significant difference has been found between females (M.Perret, unpublished data).

In the wild, females tend to stay in the locality where they are born whereas males disperse (Radesspi el et al., 2001), accounting for local competition between females for resources and mates. Social interactions between females, that usually share sleeping sites, would thus modify their hormonal pattern during the early follicular phase. An increase in local competition within females should favour the evolution of male-biased sex ratio at birth (Johnson, 1988; Chapman et al., 1989; Hewison and Gaillard, 1996; Allaine et al., 2000; Johnson et al., 2001). Previously, in captive females, excess of males at birth has been demonstrated to depend on the level of competition between females before estrus (Perret, 1990). These social effects, however, are not apparent in females experiencing their first breeding season, possibly due to the fact that the first seasonal estrous cycle of life consistently differed from the following cycles.

In humans, significant declines in sex ratio at birth have been provided in many industrial countries, though with several exceptions (Marcus et al., 1998; Parazzini et al., 1998). Adult population sex ratio appears to play a small role in determining sex ratio at birth (James, 2000) and decreases in male births have been considered to be the result of socio-economic factors (Catalano, 2003) or of increasing stressful environmental conditions (Davis et al., 1998; Astolfi and Zonta, 1999). These trends have been explained by the current idea that the production of a male birth requires good health and good social conditions (Grant, 1990; Gibson and Mace, 2003). But no significant difference in male sex ratio has been found between rural areas and polluted urban areas (James, 1998). Moreover, excess of female births occurs when women are treated for induction of ovulation by gonadotrophins (James, 1985b) whereas excess of male births has been reported during wartime (MacMahon and Pugh, 1954). Presently, changes in human sex ratio at birth might reflect the interacting effects between deterioration of environmental conditions and better medical assistance in women of industrial countries. In mammals, hormonal levels at conception affect sex ratio at birth, but in contrasting ways depending on the species and on local environmental conditions. In mouse lemurs, direction of sex ratio bias depends on hormonal stimulation during the late follicular phase, suggesting that changes in the ova environment are set prior to ovulation. This hypothesis deserves testing by assessing estrogen levels during the follicular phase and not only at conception time, in many species including humans.

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