

Birth sex ratio in the offspring of professional male soccer players: influence of exercise training load

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STUDY QUESTION: Can the exercise training load of elite male athletes influence the sex ratio of their offspring?

SUMMARY ANSWER: This is the first study assessing the influence of exercise training load on the offspring sex ratio of children from male professional athletes, observing a bias toward more females being born as a result of both high-intensity and high-volume loads, with intensity having the greatest effect.

WHAT IS KNOWN ALREADY: There is a relatively constant population sex ratio of males to females among various species; however, certain events and circumstances may alter this population sex ratio favoring one sex over the other.

STUDY DESIGN, SIZE, DURATION: Observational, descriptive cross-sectional study with a duration of 3 months.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Seventy-five male professional soccer players from First Division soccer teams. Offspring variables were sex of the offspring, number of children and order of birth. Exercise training variables were volume and intensity.

MAIN RESULTS AND THE ROLE OF CHANCE: Total offspring was 122 children (52 males (42.6%), 70 females (57.4%)). Analysis revealed that increase in either the volume ($P < 0.001$) or intensity ($P < 0.001$) of training by the players shifted the birth offspring ratio more toward females. Within the sample of females born, more births (i.e. number) were observed as a consequence of training at the highest intensity (45 out of 70; $P < 0.001$), no such pattern occurred within males ($P > 0.05$). When female versus male births were compared within each intensity, only the high-intensity comparison was significant (45 (75%) females vs 15 (25%) males, $P < 0.001$).

LIMITATIONS, REASONS FOR CAUTION: While this is the first study assessing differences in the sex ratio of the offspring of male athletes (i.e. soccer players), we acknowledge there are limitations and confounders within our approach; e.g. small sample size, ethnic background and variations in the timing of intercourse relative to ovulation as well as in sex hormone levels. As such, we propose that future research is needed to confirm or refute our findings. It is recommended that such work expand on the measurements obtained and conduct direct assessment of sperm characteristics.

WIDER IMPLICATIONS OF THE FINDINGS: The findings of the study support the fact that different stressors on the body may alter the sex of the offspring. While in the present study the stressor is the excessive training load of soccer players, other events may lead to similar results. The bias in offspring sex ratio may have important implications for demography and population dynamics, as well as genetic trait inheritance.

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Key words: male reproduction / children / sports / training volume / training intensity / exercise volume / exercise intensity

Introduction

There is a relatively constant population sex ratio of males to females among various species. For example, in humans, the World Health Organization reports on average there are 105 males (51%) born for every 100 females (49%) (World Health Organization, 2018). This ratio, however, may be affected by multitude of exogenous events and endogenous factors, and as such have important implications for demography and population dynamics (Hesketh and Xing, 2006). Among the different exogenous events, the ones most extensively studied in humans are wars (Zorn, 2002; Polasek, 2006; Helle et al., 2009), famines (Helle et al., 2009; Song, 2012), economic crises (Helle et al., 2009), exposure to chemical products-pollutants (Gomez et al., 2002; Ryan et al., 2002; Mackenzie et al., 2005; Ishihara et al., 2007; Terrell et al., 2011) and high thermal heat load exposure (Pérez-Crespo et al., 2007).

Experiencing such extremely stressful events results in a significant change in the offspring sex ratio (i.e. females > males) (Ellis and Bonin, 2004; Catalano et al., 2005; Mathews and Hamilton, 2005; Pérez-Crespo et al., 2007) and specifically in men, this shift is linked to endogenous sperm alterations (Zorn, 2002; Terrell et al., 2011) (i.e. changes in morphology, motility and quantity as a result of different chemical agents or wars) (Zorn, 2002; Terrell et al., 2011). Additionally, in animal models thermal heat stress alone results in changes in testicular spermatocytes, resulting in decreased sperm number, viability and motility, as well as sperm DNA damage (Pérez-Crespo et al., 2007). This thermal shock causes a distortion in the sex of the offspring (Pérez-Crespo et al., 2007). The common mechanism behind these sperm-related occurrences is likely the generation of reactive oxygen species (ROS) resulting in a situation of high oxidative stress and spermatogenesis disruption (Barroso et al., 2000; Aitken and Krausz, 2001). Furthermore, the Y chromosome is more vulnerable to DNA damage, and fragmenting of the DNA, than the X chromosome when oxidative stress is occurring, specifically at the time of spermatogenesis (Aitken and Krausz, 2001), and, as such leading to a bias in the resulting number of X- or Y- chromosome bearing sperm (Ellis et al., 2011; Tarin et al., 2014). Collectively, evidence supports these factors could alter the offspring sex ratio and increase the proportion of female to male births. Importantly, it should be noted that other physiological and behavioral factors can influence the offspring sex ratio (e.g. timing of intercourse relative to ovulation, coital frequency, and sex steroid hormone—gonadotrophins levels of the couple) (James 2013; James and Grech, 2018). Although relative to this last point, Hackney et al. (2016) have reported the exercise training seems to have minimal effects on the gonadotrophins.

Our research group previously found strenuous physical exercise acts as a stressor to the male reproductive system, leading to fertility problems. Specifically, alterations in sperm characteristics, such as motility, morphology, DNA integrity, viability and number all which occur as a result of excessive exercise training load, performance demands and/or certain sport disciplines (Vaamonde et al., 2006, 2009, 2014, 2018). Furthermore, we found male athletes' semen samples showed higher number of round cells and active macrophages, and increased number of germ cells, a fact suggestive of a potentially altered spermatogenesis, leading to apoptotic and necrotic events (Vaamonde et al., 2014, 2018). Such alterations in sperm morphology and DNA

fragmentation have been related to altered functionality and poor IVF success (Barroso et al., 2000; Aitken and Krausz, 2001), most likely leading to longer conception times and less possibility of successful live births (Barroso et al., 2000; Ellis and Bonin, 2004; Tarin et al., 2014). Moreover, antioxidant capacity, which helps to mitigate the effects of ROS, is decreased during intensive exercise training in men, which further accentuates these physiologic events (Vaamonde et al., 2014, 2018).

Given the above, we hypothesize that male athletes subjected to high loads of sports training at the time prior to, or at conception would suffer alterations in their offspring sex ratio from that normally expected (i.e. female > male). Along these lines, the present study analyzed the offspring sex of professional male soccer (football) athletes involved with differencing training program components (high vs low, volume and or intensity at the time of estimated conception) to assess if the male–female sex ratio at birth is altered from expected ratio levels.

Materials and methods

Study design

This was a retrospective, observational, descriptive cross-sectional study research design.

Subjects

Seventy-five professional soccer players from first division teams in South America (Chile) were recruited during the months of August to October of 2017 to participate (due to confidentiality issues team names are not disclosed). The participants were of comparable skills and socioeconomic backgrounds. In order to be eligible for the study, players had to be engaged in their usual sports activity and exercise training prior to the pregnancy of their partner; likewise, they (or their sport training staff) were required to know their training program component characteristics with regards to volume and intensity of training. The players were informed about the objective of the study and all gave their informed written consent to participate in it. This project was approved by the Ethics Committee of the University of Córdoba. Although there were not human tissue or cell samples involved, all methods were carried out in accordance with relevant guidelines and regulations, especially with regards privacy and data protection.

The selection of participants for the study was voluntary and obtained from those players who had fathered children while being engaged in sports training practice in a first division soccer team (i.e. it is important to note our sample was one of convenience).

Outcomes

The dependent variables were the sex of the offspring, the number of children and the order of birth. The independent variables were related to exercise training outcomes and consisted of the volume and the intensity of the training (i.e. training load components) at the calculated time of conception; it must be noted that soccer players do not change their training regime in a very short period of time, therefore

the information with regards training around time of conception is viewed as accurate. For each fathered child, the training load was registered. Some of the players had fathered more than one child and these events happened, while the players were undergoing different training loads.

To evaluate the training load, the total volume and intensity of training loads as well as matches-games played an interpolated time of conception (40 weeks before delivery) was utilized as the point of reference. Soccer as a sport has great variability in training/activity patterns at different time points during the season, depending on the timeline of training development and match importance. For this reason, it was considered that athletes accumulated low workloads (low volume and low intensity) when they were in rest period (transitory period), a medium load (low volume and moderate or low-moderate intensity) when they were competing in the championship (competitive period) and, finally, it was considered as a high load (high volume and high intensity) to the work done by the players during the preparation phase before the league matches (pre-season). These criteria were based upon the research work on soccer training loads reported by Bangsbo (1999). Specifically, training volume and intensity were quantified as follows: low volume: less than 4 training sessions/week (sessions being 1–2 h of physical training); moderate volume: 6–8 training sessions/week; high volume: >8 training sessions/week. Intensity was quantified numerically using the Borg scale of perceived exertion from 1 to 10 (Borg, 1998): low intensity: perception of effort (PE) <6; moderate intensity: PE 6–8; high intensity: PE >8. As exclusion criteria, potential participants were not considered if: any fertility alterations existed in the player or partner, the player had children from more than one partner, the player did not have any offspring's at the time of the study or if they had a premature child (i.e. the latter makes it complex to estimate the date of conception), or the offspring came from a multiple pregnancy (twins/triplets, etc.).

Both the players and their partners conceived their children at an age that is considered as healthy from a reproductive point of view (~20 to ~30 years of age) (men specifically were; age = 29.6 ± 5.5 years, body mass = 76.9 ± 6.5 kg, height = 177.2 ± 7 cm (mean \pm SD)). Furthermore, the player's partners were not athletes and did not engage in sports training.

For data collection, the participants completed a self-administered reproductive health questionnaire during a personal meeting in which an investigator explained the objective and the procedures of the study and thereby, making it possible to resolve any doubts regarding the questionnaire responses. The questionnaire had several items regarding lifestyle habits of player and partner just to rule out potential confounders. The process was carried out jointly with the fitness trainer or other members of the technical staff of the sports organization with regards to training components, making sure data regarding the training components was accurate.

Sample size estimation

Considering the absence of any previous data on the association of training load and bias in the offspring sex ratio, we used data generated from our previous work (Vaamonde *et al.*, *in press*). The calculation of the sample size was made based on a difference in intensity

percentage among groups reported within this latter study. A confidence level of 95% and a statistical power of 80% was used. From these calculations, a minimum sample size of 68 participants was deemed to be appropriate.

Statistical analyses

The sex of the offspring was taken as the main dependent variable. Training load expressed as volume (low, moderate, high) and intensity (low, moderate, high) of exercise were the major independent variables. Other variables analyzed were: the number of children of each athlete (which ranged from 1 to 4), order of births and existence of some problem in the fertility pattern (specifying it with each son or daughter).

To examine the qualitative variables, frequency tables were used, and to relate the variables of volume and intensity training load, specifically with the offspring variables. To this end, the χ^2 test was used and probability level for statistical significance was set as $P < 0.05$. Fisher's exact test was used for the high-training load versus low-training load analysis. The statistical analysis was carried out using the SPSS[®] 22.0 program.

Results

Seventy-five soccer players who fathered a total of 122 live child births participated in the study. One of the players was excluded as he had fathered three children from three different partners. None of the children included in the analysis came from a multiple pregnancy (twins/triplets). The overall total offspring ratio was 52 males (42.6%), and 70 females (57.4%).

As noted, training volume and intensity were each stratified into low, moderate and high categories. Differences in both the volume ($P < 0.001$) and intensity ($P < 0.001$) of training influenced the observed sex of the child. Specifically, the number of females being born was significantly increased with respect to the number of males when the fathers engaged in training involving either a high volume or high intensity compared to respective lower volume or intensity training periods (see Table I).

Relative to intensity, when examining just the female children sample, significantly more births of girls were observed in the period of training that involved high-intensity exercise versus moderate or low intensities (i.e. 45 vs 12 vs 13 out of 70, respectively; $P < 0.001$; see Table I). No such pattern was found in the sample of male children (15 vs 23 vs 14 out of 52, $P > 0.05$). As noted, when female versus male births were compared within each intensity, only the high-intensity comparison was significant (45 (75%) females vs 15 (25%) males, $P < 0.001$).

Interestingly, when examining a subgroup of offspring ($n = 53$) in which their athlete fathers engaged in periods of both combined high-load volume + high-load intensity training, versus a low-load volume + low-load intensity training, an even slightly greater bias toward girls birth was detected ($P < 0.05$; Table II).

There were no associations between the sex of the child and the order of child birth ($P > 0.05$; Table III).

Table I The number (and percentage within an individual sex) of children born relative to the influence of the individual training factors (volume and intensity) monitored in the study.

Volume training component					
Boys			Girls		
Low	Moderate	High	Low	Moderate	High
22 (42.3%)	23 (44.2%)	7 [†] (13.5%)	32 (45.7%)	2 (2.9%)	36 [†] (51.4%)
$P < 0.001$					
Alike symbols differ					
Intensity training component					
Boys			Girls		
Low	Moderate	High	Low	Moderate	High
14 (27.0%)	23 (44.2%)	15 [†] (28.8%)	13 (18.6%)	12 (17.1%)	45 [†] (64.3%)
$P < 0.001$					
Alike symbols differ					

Table II Number of male and female children born from male soccer players undergoing either high-load training involving both high volume (V) + high intensity (I) versus low-load training involving low volume + low intensity ($P < 0.05$, alike symbols differ).

Sex	High-load (high V + high I)	Low-load (low V + low I)	Total
Girls	24 [‡] (80%)	12 (52%)	36
Boys	6 [‡] (20%)	11 (48%)	17
Total	30	23	53

Percentages represent within a respective training load.

Table III The order of childbirth does not associate with off-spring sex ratio.

		Order of child birth				Total
		1st	2nd	3rd	4th	
Sex	Boy	36	14	2	0	52
	Girl	39	19	11	1	70
Total		75	33	13	1	122

Values are number of children born (no significant differences were noted due to order $P > 0.05$).

Discussion

To the best of our knowledge, this is the first study that assesses the influence of exercise-sports training load on the sex of the offspring of male soccer athletes. The results reveal a significant bias in the sex ratio toward females as a result of higher-load volume and, or intensity of training. The observed bias toward greater female offspring is in opposition to the general statistical data for sex ratio of live births in the region to which the teams belonged which is consistently around 51% males (Anuario de Estadísticas Vitales, 2015, 2017) as well as worldwide (World Health Organization, 2018). We postulate the bias in the sex of the offspring seems to be a consequence of high-load training (volume and/or intensity) acting as a physiological stressor to the male reproductive system by potential means of several interrelated pathways; such as increased core temperature, increased glucocorticoid production and increased ROS-oxidative stress (Vaamonde et al., 2006, 2009, 2014, 2018). Regarding the latter, it is accepted that the Y chromosome is more vulnerable to stress damage than the X chromosome, hence being affected to a greater extent by such stressor events, and as such potentially leading to the ratio deviation observed (Aitken and Krausz, 2001).

Soccer is an intermittent type sport when matches are played, characterized by periods of activity and inactivity and as such of varying degrees of physiological stress (Smpokos et al., 2018). Player activity relies on anaerobic actions (i.e. sprints), but also greatly on aerobic metabolism (matches are 90 min) and these metabolic demands involve elevated consumption of glycogen reserves as well as inducing fat oxidation (Hulton et al., 2013). In order to prepare for these games, players need to undergo training loads involving high-volume and high-intensity efforts. The high metabolic demands of this sport promote ROS formation because of the imbalance between oxidant/antioxidant status of lymphocyte and neutrophil populations (Sureda et al., 2009). While an accumulative effect cannot be ruled out, some of the existing reports on the effect of exercise on sperm characteristics show clear acute effect (Vaamonde et al., 2006); therefore, periods of high volume and high intensity would be more detrimental to sperm.

The X- and Y- chromosome bearing sperm respond differently to different stressors that can damage sperm DNA (Pérez-Crespo et al., 2007; Oyeyipo et al., 2017; You et al., 2017; Shi et al., 2019), and, specifically the spermatocyte and spermatid stages of development are more susceptible to such damage (Pérez-Crespo et al., 2007; Malo et al., 2017). This results in distorted proportions of the sex chromosomes during these spermatogenic processes (Banks et al., 2005). While apoptotic events eradicate some of the defective sperm, some damaged cells do complete the spermatogenic cycle (Malo et al., 2017); even though they exhibit differences in survival or fertilizing capacity (Ishihara et al., 2009).

Intense exercise has been linked to several altered semen parameters (Vaamonde et al., 2006, 2009, 2014, 2018) as well as increased sperm DNA fragmentation, increased presence of macrophages, increased ROS and a decreased total antioxidant capacity (Vaamonde et al., 2014, 2018). It is clear that the type of sports training, athletic performance level and the physical exercise effort can potentially modulate these reproductive responses (McMurray and Hackney, 2000) and as such may impact the specific time point of conception.

We recognize there are limitations (e.g. small sample size, a sample of convenience) and potential confounders to our study, such as ethnic background, parental age, socioeconomic status, timing of intercourse relative to ovulation, coital frequency and sex steroid hormone—gonadotrophins (in either parent) levels all of which can impact on the offspring sex ratio (James 2013; James and Grech, 2018). Thus, we acknowledge further research is needed to confirm or refute our findings which must be viewed as preliminary. It is recommended that such future work expand on the measurements obtained, implement a design that overcomes our sampling limitations, and most specifically conducts direct assessment of sperm characteristics and hormonal profiles.

In conclusion, high-level sports training should be considered as a stressor to the reproductive system, and as such may potentially skew the sex at birth of the offspring; i.e. affecting population dynamics. Further studies are needed to confirm our sex bias findings. It is also necessary to clarify the underlying mechanisms leading to this bias so a better understanding as how the primary sex ratio is modulated.

Author's roles

Design: D.V., A.C.H., J.M.G.M.. Literature search: D.V., A.C.H., J.M.G.M., E.A.A., M.V. Sample and data collection: E.A.A., J.M.G.M.. Statistical analysis: M.V. Manuscript preparation: D.V., A.C.H., J.M.G.M., M.V.

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Conflict of interest

The authors have nothing to disclose.

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