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Evidence-based practice

Spontaneous recovery from reproductive failure in a hand-reared male western lowland gorilla (*Gorilla gorilla gorilla*)

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Abstract

An adult hand-reared male gorilla raised in bachelor groups was considered as not reproductive on the basis of his behavioural background, clinical history and the results of a physical examination (electroejaculation and ultrasound examination of the testes). He was kept for 3.5 years with a well-socialised adult female and, despite regular sexual activity, no reproductive success was achieved. However, this gorilla has since returned to fertility and within a few months of a second female joining the group, both females became pregnant, indicating that the male could no longer be deemed "infertile". Thus, male fertility evaluations must be interpreted carefully in relation to social structures in gorillas, and the establishment of a proper social group structure should be taken into consideration when establishing new gorilla breeding groups. The introduction of adequately socialised females to hand-reared males that have matured in bachelor groups may improve not only the social behaviour but also the reproductive capacity of such males.

Introduction

Success in the captive breeding of gorillas in European zoos has improved in the last decade and the population is increasing slowly (Abello et al. 2010). However, there is still a considerable number of non-breeding animals. Hand-reared males, raised in bachelor groups, are of particular concern as they are less successful as breeding animals (Abello et al. 2010, 2011). Nevertheless, there is little available information in the literature concerning ongoing monitoring of the reproductive success of these animals. The aim of the present report is to document the reversibility of infertility in a hand-reared male gorilla, highlighting that male fertility evaluations must be interpreted carefully in relation to social structure in gorillas.

Methods

A 16.5-yr-old male western lowland gorilla ("Mambie"), born in March 1991, was transferred on 14 December 2007 to Bioparc Valencia (Spain) as the breeding male for a new breeding group. The animal was hand reared and kept in a sibling group at his natal zoo, and later transferred to two different bachelor groups in two further zoos at the ages of eight and 11 years. Thus, he did not have any mating experience or the chance to observe mating in other conspecifics. On the same day an 8-yr-old mother-reared female gorilla ("Fossey") also arrived from a different zoo. This female had been parent reared in her natal group, was an adequately socialised animal, and should therefore have supposedly had a positive effect on the male by improving his social skills. The introduction of the gorillas proceeded without any difficulties within a few days. Both animals were considered sexually mature according to their age (De Vries and Glatstone 2005; Abello et al. 2010). Despite the limited social behaviour of the male, both animals were considered compatible and copulated regularly after their arrival, although no pregnancy was achieved. As previously mentioned, hand reared males raised in bachelor groups have particularly poor breeding results. Thus, on 4 June 2009, 1.5 years after the arrival of the male, a thorough physical examination of the male was carried out under general anaesthesia.

The ultrasonographic examination was performed with a GE Voluson 730 Pro equipment (General Electric Medical Systems, Kretzetchnik Gmbh co., 4871 Zipf, Austria) with a linear multi-frequency probe at 10 MHz for testicular examination. The bladder was emptied of urine and subsequently rinsed with

20 ml Human Tubal Fluid (HTF, LifeGlobal, Barcelona, Spain) in order to recover the retroejaculated sperm from the bladder. A rectal probe was inserted into the rectum and a series of low level electrical stimulations were delivered to the prostate gland and seminal vesicles via direct stimulations of the pelvic floor using a LifeSTIM™ System electroejaculator (Neurocontrol Seager, Ohio, Cleveland, USA). A total of eight stimuli of approximately five seconds were applied, starting at a voltage of five volts and increasing the voltage stepwise to a maximum of 25 volts and 600 milliamperes of current. The ejaculate was evaluated according to standard procedures as described for humans (WHO 2010).

Results

The 150 kg gorilla was found to be in excellent general condition. The ultrasonographic examination of both testicles showed diffuse microcalcifications throughout the testicular parenchyma (Figs 1 and 2). Testicular measurements (length x width) of the right and left testis were 37 x 16 mm and 39 x 17 mm respectively, within the published range obtained from reproductively successful gorillas (Schaffer et al. 1981). Approximately 0.3 ml of fluid was recovered during electroejaculation, which was then combined with the medium recovered by means of bladder catheterisation after electroejaculation. Only three sperm cells with progressive motility were found after an extensive search in multiple fields of centrifuged pellets. Thus, although the total ejaculate volume is not known, we may estimate the sperm concentration to be less than 0.1 x 10^6/ml. Hormone analysis included follicle-stimulating hormone (FSH; 11.95 IU/l), luteinising hormone (LH; 4.72 IU/l) and testosterone (4.2 ng/ml). An additional blood sample collected 6 months later during a minor procedure showed an FSH of 9.61 IU/l.

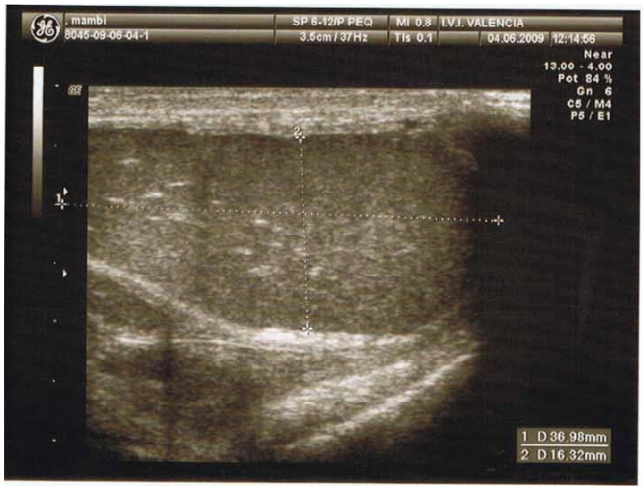

Figure 1. Sagittal section of the right testicle showing "starry sky" appearance due to microcalcifications revealed by the ultrasound. Sagittal and transverse (not showed) sections of the gorilla's right testis revealed several microcalcifications throughout the testicular parenchyma. Volume = 4.7 cc; size = 37 x 16 mm.

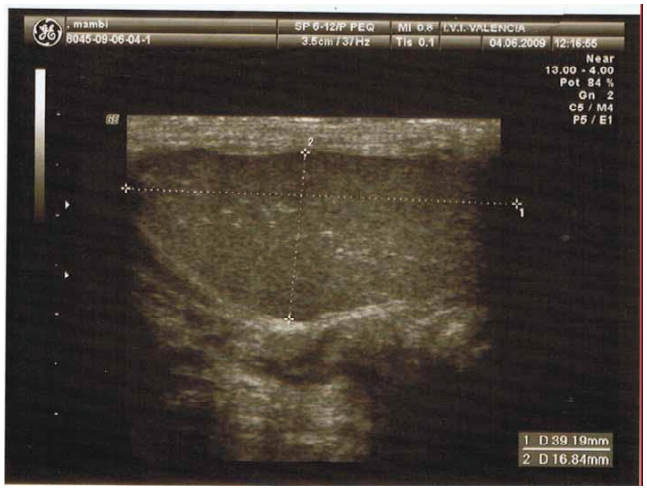

Figure 2. Sagittal section of the left testicle showing similar but less severe appearance due to microcalcifications revealed by the ultrasound. Volume = 5.6 cc; size = 39 x 17 mm.

On 29 March 2011, 2 years and 3 months after the arrival of the first pair of gorillas, an additional 10-yr-old mother-reared female gorilla ("Ali") was added to the group. After a slow and progressive introduction process of two months, all three animals were finally housed together from 26 May 2011. Behavioural records collected on this day noted that the male displayed a complete silverback sequence towards the females, starting with soft clear hoots, then running bipedally sideways, throwing objects into the air and finally beating his chest. Both females retreated to a safe distance during the displays and were submissive towards the silverback, particularly the recently arrived female. A full silverback display towards other gorillas had never before been observed since the arrival of the male in Valencia. Ten months later, on 3 April 2012, the first female gorilla ("Fossey") gave birth to a normal baby**.** The average length of gestation for gorillas in captivity lasts 255 days (De Vries and Glatstone 2005), and fertilisation must therefore have taken place during July 2011, some five to nine weeks after the third gorilla was introduced. In fact, copulations were recorded on 18 and 25 July 2011. The second female was also fertilised by the male in February 2012.

Discussion

The microcalcifications noted in the testicular parenchyma are described in human medicine as testicular microlithiasis (TM) (Yee at al. 2011). It is a relatively uncommon condition, and can be found in 0.6–9% of men referred for testicular ultrasound, although the true incidence in the general human population is unknown (Dohle et al. 2005). In a recent study, TM was diagnosed in 87 patients (6%) out of a total of 1439, and a significant co-ocurrence of TM, testicular cancer and infertility was noted (Yee at al., 2011). Its relationship with infertility is unclear, but probably relates to dysgenesis of the testis (Dohle et al. 2005; Yee at al. 2011). To our knowledge, TM has not yet been described in gorillas, and its significance in this species is unknown.

Standard semen characteristics alone are an indirect measure of sperm function and are not diagnostic of fertility (Spindler and Wildt 2010). However, although the minimum ejaculate characteristics required to produce a pregnancy in gorillas are unknown, this sample is far below the normality thresholds in gorillas and humans (Gould 1983; Sikaris et al. 2005), with an extremely low possibility of being able to achieve parenthood. Reference values for sperm concentration in gorillas range between 15 and 360 x 10^6 (Gould 1983). Testosterone values for mature gorillas ranged between 3.450 and 7.158 ng/ml (Gould 1983). Although we are not aware of reference data for pituitary hormones in gorillas, we used the reference intervals for FSH (1.3–8.4 IU/l) and LH (1.6–8.0 IU/l) published for humans (Sikaris et al. 2005), as gonadal and

pituitary hormone values for fertile adult male apes fall within the normal range for humans (Gould 1983). The hormones were considered within normal limits in this case, except for FSH, which remained elevated 6 months later. An elevated FSH value is an indication of damaged or dysfunctional spermatogenesis in both humans and gorillas (Gould 1983; Sikaris et al. 2005). Although the doses of anaesthetics applied were within the recommended range for this species (Sleeman 2007), it has also been noted that some anaesthetics might inhibit ejaculation (Spindler and Wildt 2010). Nevertheless, by the time of this examination, the male had been copulating regularly with the female for 1.5 years but no pregnancy was achieved.

The etiology of infertility is often obscure. In 40–60% of cases of infertile men, the only abnormality is found in the semen analysis and there is no relevant history or abnormality on physical examination and endocrine laboratory testing (Dohle et al. 2005). Also, spontaneous recoveries of fertility have been reported in couples with male infertility factor after discontinuation of assisted reproductive techniques (Osmanagaoglu et al. 2002).

Histological examinations performed on the testicles of male gorillas kept at Japanese zoos provided evidence for spermatogenesis in only 40% (n=10) of the samples analysed (Enomoto et al. 2004). In another study, spermatogenesis was evident in 100% (n=7) of the testicles of orangutans and 91% (n=11) of the testicles of chimpanzees also kept in Japanese zoos under similar conditions to those of the gorillas (Fujii-Hanamoto et al. 2011). Thus, spermatogenesis in captive gorillas appears to be more vulnerable in comparison with other great apes.

The total duration of spermatogenesis in most mammals lasts nearly 40 to 54 days (Hess and de Franca 2008). However, in great apes this process is longer, and lasts more than 70 days in humans (Hess and de Franca 2008), and 62.5±1.5 days in chimpanzees (Smithwick et al. 1996). To our knowledge, this process has not been studied in gorillas, but it is reasonable to expect a similar duration. Between the onset of the typical silverback behaviour and the estimated fertilisation date there is a period of around two months during which an entire new spermatogenic cycle may have been completed.

The remarkable gorilla display is a key communicatory signal in silverbacks. Agonistic displays by male western lowland gorillas in the wild are particularly intense in nature towards immigrant females, and are considered as “courtship aggression”, which is an important part of the mating strategy of this species (Stokes 2004). Thus, it might be speculated that the addition of the second female to the gorilla pair provided sufficient social stimulation and finally triggered spermatogenesis. Spermatogenic arrest in gorillas may be corrected by alterations in their environment and management (Gould 1983). Also, social factors, such as the removal of a certain female, have been associated with the sudden decrease in the sperm quality of a proven breeder (Schaffer et al. 1981). In this particular case, the introduction of an additional female was the only factor changed, which finally resulted in two pregnancies within eight months with two different females after 3.5 years of reproductive failure, even though active copulatory activity with intromission was observed on a regular basis. This strongly suggests that his sperm quality must have improved sufficiently to have fathered offspring twice.

Spermatogenesis is a complex and species-specific biological process of cellular transformation, and is dependent upon numerous factors. In highly intelligent and social animals, such as great apes, social influences also play a major role. Western lowland gorillas in the wild live in cohesive, predominantly single-male groups with an average of 3.8 females per group (Stokes 2004). Keeping males of polygynous species such as gorillas in pairs may therefore have detrimental effects on their reproductive function, and this should be taken into consideration when establishing new gorilla breeding groups. The introduction of adequately socialised females to hand-reared gorilla males that have matured within bachelor groups may improve the social behaviour of the males, but may also have a stimulatory effect on their spermatogenesis and reproductive capacity.

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