ASPECTS OF THE REPRODUCTIVE PHYSIOLOGY OF MALE VERVET MONKEYS MAINTAINED IN A LABORATORY ENVIRONMENT

by

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I, Jürgen Victor Seier, declare that the "ASPECTS OF THE REPRODUCTIVE PHYSIOLOGY OF MALE VERVET MONKEYS MAINTAINED IN A LABORATORY ENVIRONMENT" is my own work and that all sources I have used or quoted have been indicated and acknowledged by means of complete references.

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Dedicated to my wife Sally

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Abstract

In biomedical research limited use has been made internationally of African non-human primates. As a result, their reproductive physiology has been less well defined, when compared to the more commonly used macaque species. There have also been no extensive developments in the associated field of assisted reproduction for African non-human primates.

To contribute to the knowledge of the reproductive physiology of African non-human primates, this study presents semen characteristics from the vervet monkey. The focus is on abnormal sperm morphology which has not been described in detail. All individuals utilized were either wild caught or colony bred and maintained in an indoor laboratory breeding colony.

A detailed description of the frequency and prevalence of specific morphologically abnormal forms of sperm from vervet monkeys and illustrations of the different types of abnormalities is provided for the first time. Most features, such as the prevalence of tail abnormalities, particularly coiled and bent tails, were similar to what has been reported for other Old World cercopithecines. A total of 28 types of morphologically abnormal forms were found including 13 head abnormalities, seven midpiece abnormalities and eight principal- and endpiece abnormalities.

Sperm head abnormalities were rare, occurring at a rate of less than 2% in each group. Except for the nipple defect, there was no difference between colony bred and wild caught individuals in the rate at which defective forms occurred. On the other hand not all types of abnormalities were found in each group.

A relationship between morphology and fertility could not be established because some individuals with the highest rate of abnormal morphology were successful breeders. Evaluation of consecutive ejaculates revealed highly variable semen characteristics both within and amongst different individuals. This observation extends to other sperm characteristics such as the concentration, vitality, speed of forward progression (FP) and motility. The evaluation of reproductive potential based on one or two ejaculates is therefore not possible.

The pH of vervet monkey semen was similar to that of humans. Out of two indicator papers tested to determine this variable, only one was found to be reliable in terms of reference values obtained with a pH meter.

Another aim of the study was to develop a cryopreservation method which yields a satisfactory post-thaw recover of progressively motile sperm. No specialized equipment other than a liquid nitrogen storage container was needed. Semen samples were diluted with a Tes-Tris extender containing 5% glycerol before being cooled to 5° over 30 minutes.

This was followed by aspiration of the samples into paillettes and freezing to a plunge temperature of -139°C for 20 minutes in liquid nitrogen vapour. The paillettes were finally stored in liquid nitrogen. Conspicuous features of this method were the addition of glycerol at 32°C, a short glycerol equilibration time, a fast freezing rate, a low plunge temperature and a fast thawing rate.

Opsomming

In biomediese navorsing op internasionale gebied, is die gebruik van Afrika-nie-menslike-primate beperk. Derhalwe is hul voortplantingsfisiologie minder deeglik gedefinieer as dit vergelyk word met die mees algemeen gebruikte *Macacca* spesies. Daar is ook geen uitgebreide ontwikkelings in die geassosieerde veld van geassisteerde reproduksie vir Afrika-nie-menslike-primate nie.

Om by te dra tot die kennis van die voortplantingsfisiologie van die Afrika-nie-menslike-primaatspesies, verstrek hierdie studie die semeneienskappe van die blouaap. Die fokus is op abnormale spermmorfologie, wat tot nog toe baie min aandag geniet het. Die diere vir die studie is onderhou in 'n binnenshuise laboratorium-teelkolonie en bestaan uit individue wat in die natuur gebore is, sowel as diere wat in gevangeskap gebore is.

Die voorkomsyfer en frekwensie van spesifieke morfologies abnormale vorms van blouaap sperme, word beskryf en geïllustreer vir die eerste keer. Die meerderheid van kenmerke soos die voorkoms van stertabnormaliteite, veral gekronkelde en geboë sterte, was soortgelyk aan die wat gerapporteer is vir ander ou-wêreld *Cercopithecidae*. 'n Totaal van 28 morfologies abnormale vormtipes is gevind en bestaan uit 13 kopabnormaliteite, 7 middelstukabnormaliteite en 8 hoof- en

endstukabnormaliteite.
Spermkopafwykings was skaars, met 'n voorkomsyfer van minder as 2% in elke groep. Met die tepelafwyking buite rekening gelaat, was daar geen verskille in die tempo waarteen abnormale vorme voorgekom het tussen koloniegeteelde en natuurgebore individue nie. Nie alle tipe abnormaliteite het egter by elke groep voorgekom nie.

'n Verwantskap tussen morfologie en vrugbaarheid kon nie gevind word nie, as gevolg van die feit dat van die individue met die hoogste abnormale tellings, goed geteel het. Ontleding van opeenvolgende ejakulate het groot variasie in semeneienskappe tussen monsters van dieselfde individue en ook tussen verskillende individue getoon. Hierdie bevinding geld ook vir ander spermeienskappe soos konsentrasie, lewenskragtigheid, spoed en voorwaartse beweging en beweeglikheid. Dit is daarom nie moontlik om voortplantingspotensiaal te evalueer op een of twee ejakulate nie. Die pH van die blouaap semen, was soortgelyk aan die van die mens en slegs een van die twee tipes toetsstrokies, wat vir hierdie doel gebruik is, het waardes weerspieël wat vergelyk het met pH-meterlesings.

Nog 'n rede vir hierdie studie was om 'n vriesbewaringsmetode vir blouaapsperme te ontwikkel, wat 'n aanvaarbare na-ontdooiingsopbrengs van sperme met 'n voorwaartse beweeglikheid sou gee. Die enigste spesiale apparaat wat hiervoor benodig was, is 'n bergingshouer met vloeibare stikstof. Semenmonsters is verdun met 'n Tes-Tris verdunningsvloeistof wat 5% gliserol bevat, voordat hulle afgekoel is tot 5°C, oor 'n periode van 30 min. Hierna is die monsters opgetrek in strooitjies en afgekoel vir 20 min in vloeibare stikstofdamp tot 'n voor-onderdompelingstemperatuur van -139°C. Na onderdompeling word die strooitjies so bewaar. Uitstaande kenmerke van hierdie metode is die byvoeging van gliserol by 32°C, 'n kort gliserol-balanseringstyd,'n vinnige vriestempo, 'n lae dompeltemperatuur en 'n vinnige ontdooiingstempo.

CHAPTER I

1. General introduction and background

The mammalian order of primates is regarded as the highest evolved in terms of brain size and complexity and cognitive abilities (Ankel-Simmons 1983 a, Fobes and King 1982). Both human and non-human primates belong to this order and many behavioural, genetic, anatomical and physiological similarities reflect this close phylogenetic relationship (Hendrickx and Binkerd 1990, King *et al*. 1988, Müntzing *et al*. 1975, Smith and Williams 1974). As a result, non-human primate species are often regarded as the most relevant and important animal model in biomedical research (Hendrickx and Binkerd 1990, King *et al*. 1988, Lapin 1982, Müntzing *et al*. 1975, Smith and Williams 1974).

The similarities are particularly conspicuous when comparing the reproductive physiology and anatomy of human and non-human primates (Ankel-Simmons 1983 b, Hendrickx and Binkerd 1990, King *et al*. 1988, Müntzing *et al*. 1975). These include obvious features such as the menstrual cycle, a simplex uterus and a pendulous penis (Ankel-Simmons 1983 b). But there are many other similarities such as aspects of the endocrinological control of the reproductive cycle and pregnancy, implantation, placentation and embryogenesis, male accessory gland function, spermatogenesis and spermiogenesis (Ankel-Simmons 1983 b, Hendrickx and Binkerd 1990, King *et al*. 1988, Müntzing *et al*. 1975, Tanimura and Tanioka 1975).

Detailed definition and understanding of the reproductive physiology of primates, which is only possible by maintaining captive populations, is necessary for three main reasons:

1. To support the management of wild populations in their natural habitats and for a complete understanding of their reproductive biology and phylogenetic position.

2. It is essential for the effective breeding management of captive non-human primates. Effective breeding is supported by the development of associated techniques for assisted reproduction such as cryopreservation of semen. Captive propagation is regarded as the only ethical alternative to ensure the future supply of primates for biomedical research and to preserve the gene pool of rare and or endangered species.

3. Reproductive research utilizing primates needs to be based on defined models and detailed baseline data.

The primate species most commonly utilized in biomedical research are from the genus *Macacca*, all of which except for one species occur in Asia (Held and Wolfle 1994, King *et al.* 1988). The reasons for this preference are historical rather than biological or scientific. Consequently the reproductive physiology of members of this genus has been extensively studied and is well defined (Austin 1975, Barnes *et al.* 1978, Goodman *et al* 1977, Gulyas *et al.* 1976, Hein *et al.* 1989, Mahoney 1975, Monfort *et al.* 1986, Moudgal 1984, Shackleton and

Mitchell 1975, Tanimura and Tanioka 1975, Wehrenberg *et al.* 1979).

Limited use has been made internationally of African primate species, resulting in a particular lack of data of aspects of male reproductive physiology. The reason is that the study and definition of the reproduction of non-human primates has often been a by-product of contraceptive research. This frequently targets the female and uses the male only as a sperm donor. Since the vervet monkey is indigenous to Southern Africa, it is one of the two primate species of choice in local biomedical research. Because of it's size it is, however, the only species with the potential of complementing or substituting the internationally more commonly used macaques. Yet little contribution has been made locally to the study and definition of vervet reproduction or the development of techniques for assisted reproduction.

The following is an introduction to the vervet monkey's natural history and breeding biology and a review of vervet monkey and general non-human primate spermatology. Since the focus will be on sperm morphology and the cyropreservation of semen some basic principles are reviewed and discussed in both fields.

1.1 The vervet monkey

1.1.1 Natural history

The vervet monkey (*Cercopithecus aethiops*), also called African green monkey, is one of four primate species indigenous to Southern Africa (Napier and Napier 1967). Taxonomically it is one of about 20 species and over 60 subspecies of the genus *Cercopithecus* (Fobes and King 1982, Napier and Napier 1967). This genus together with previously mentioned *Macacca* and *Papio* species belong to the same family, the *Cercopithecidae* (Fobes and King 1982, Napier and Napier 1967). All are categorized into the infraorder Old World primates (*Catarrhini*) and the mammalian order of primates (Fobes and King 1982).

Being widely distributed throughout sub-Saharan Africa and often abundant where it occurs, it's habitat include most areas except deserts, open grasslands, dense forests and mountains (Eley 1992, Napier and Napier 1967). The vervet monkey is an omnivorous feeder and lives in small multi male multi female groups of up to 50 to 60 individuals. Groups are socially highly organised with a linear dominance relationship amongst the males and a matriarchal kin group relationship amongst the females (Eley 1992). The body weight of an adult male ranges from 4 to 7 kg and that of a female from 3 to 5 kg. Vervets can lead an arboreal and terrestrial existence which has made them so successful in terms of distribution.

1.1.2 General breeding biology

The vervet monkey has been described as a marginal seasonal breeder but breeds all year round in captivity (Eley 1992, Seier 1986). The females experience no estrogen stimulated cyclic perineal swelling which is the hallmark of cyclicity of many other primate species (Seier *et al.* 1991). Males and females mate throughout the cycle and not just pre-or periovulatory which, according to one hypothesis, is typical for species without cyclic perineal swelling. Normally one infant per year is produced which stays for about 12 months with it's mother.

1.2 Review of Vervet male reproductive physiology with a focus on spermatology and cryopreservation of semen

1.2.1 *Spermatogenesis, spermiogenesis and semen biochemistry*

The cellular associations and morphological features during spermatogenesis and spermiogenesis have been described for vervet monkeys by Barr and Clermont (1973, 1969). The former author also defined the duration of spermatogenesis and the length of one seminiferous cycle (Barr 1973). The structure of Leydig cells is reported to follow the general pattern found in other mammals (Camatini *et al.* 1981). Defective spermatozoa are removed by phagocytosis from the epididymis (Roussel *et al.* 1967).

No seasonal changes in seminiferous tubule diameter nor changes in spermatogenic activity suggesting seasonality were reported from captive vervets from East Africa (Eley *et al.* 1986).

A number of biochemical parameters studied in vervet semen including fructose, lactic acid and citric acid were within the range of many other non-human primate species (Ackermann and Roussel 1968). The same authors also investigated the citric acid, lactic acid and oxygen metabolism of fresh and frozen vervet semen (Ackerman and Roussel 1971).

Testosterone concentrations in adult male vervets are similar to those in rhesus monkeys (Eley *et al.* 1986) and no seasonal changes were recorded. However diurnal fluctuations of androgen and estradiol concentrations were recorded in adult male vervets (Beattie and Bullock 1978).

1.2.2 *Spermatology of the vervet monkey*

Most studies on vervet semen were confined to investigating sperm concentrations and motility. Roussel and Austin (1967) studied, amongst others, the motility of sperm from five non-human primate species. A mean sperm motility of 53% was recorded for eight samples from three vervet monkeys. This was similar in the other four non-human primate species investigated in the same study. A more detailed spermiogram of 11 primate species was provided by Ackermann and Roussel (1968). Six ejaculates from two individual vervet monkeys were evaluated according to four parameters. The sperm concentration was very variable ranging from 57 million/ml to 288 million/ml and the motility varied from 34 to 38%. The percentage of vital spermatozoa was 45% and 50% as determined with eosin staining. The spermatozoa with abnormal morphology were reported to be 26% and 40% but the different abnormal forms, their rate and prevalence were not investigated. In another study the sperm concentration and motility of seven semen samples from two vervet monkeys were determined (Ackermann and Roussel 1971). The concentration of 1428 million/ml and extremely low motility of 1.1% was not in agreement with any of the other values reported previously.

Another more complete spermiogram was provided by Valerio and Dalgard (1975). This included for the first time the ejaculated volume which was 1.24ml and was made up of a liquid portion of 1ml and a coagulum of 0.24ml. The sperm concentration was found to be 144 million/ml, the motility 58% and spermatozoa with abnormal morphology 24%. Again no specific defects, their prevalence or rates at which they occur were reported. The ratio of dead sperm was 13%, which was lower than reported previously for vervet monkeys. The sample size for this study was not mentioned. Hendrickx *et al.* (1978) reported some semen characteristics of 23 ejaculates from four vervet monkeys. The average ejaculate volume was reported to be 0.9 ml, the mean sperm concentration 440 million/ml and the motility was 39%. These results reflected again the big variability in the reported semen characteristics of vervet monkeys. In a study to investigate the influence of ethanol on the semen characteristics of six adult males, the sperm concentration and gross normal morphology were determined (Van der Colf *et al.* 1991). The sperm concentration agreed mostly with what was reported in above mentioned studies. Few defective spermatozoa were found and the gross normal morphology varied mostly between about 67% and 100%. No reference was made to specific defects.

The only mention of a specific morphological defect in ejaculated semen in vervet monkeys was made by Conradie *et al.* (1994). The acrosomal integrity was studied in fresh spermatozoa and it was found that an average of 61.2% were intact, 18.4% mildly damaged, 16.8% severely damaged and 4.6% lost or reacted acrosomes. The work was done on 10 semen samples from 10 different monkeys.

Despite the big variation of results, vervet monkey sperm characteristics were similar to those of other *Cercopithecines*. However, nobody has provided a detailed description and or illustration of the type of abnormal forms, the rate at which they occur and their prevalence. The pH is another semen characteristic that has not been investigated.

Although the influence of abnormal morphology on fertility has not been established in non-human primates, a definition is necessary for a complete understanding of vervet spermatology and as baseline data from which to evaluate changes in the semen profile. This will assist in the investigation of reproductive potential, disease states, and the influence of compounds on the male reproductive system during pharmakodynamic and general toxicological studies.

1.3 General primate spermatology

The viability of ejaculated spermatozoa are usually evaluated by establishing a spermiogram using standard techniques and parameters (World Health Organisation 1992). Spermiograms have been established for a number of primate species and the most frequently reported parameters are: sperm concentration, motility, vitality and the proportion of total abnormal or normal morphology (Ackermann and Roussel 1968, Bornman *et al.* 1988, Bush 1975, Gould and Mann 1988, Harrison *et al.* 1986, Harrison and Wolf 1985, Harrison 1980, Hendrickx *et al.* 1978, Sarason *et al.* 1991, Schaffer *et al.* 1992, Thomson *et al.* 1992 Valerio and Dalgard 1975, Lang 1967).

The different morphologically abnormal forms which occur in most ejaculates and the rate at which they occur appears not to have been studied in detail and most investigators report only the total normal or abnormal morphology (Ackermann and Roussel 1968, Bornman *et al.* 1988, Bush *et al.* 1975, Gould and Mann 1988, Harrison *et al.* 1986, Harrison and Wolf 1985, Harrison 1980, Hendrickx *et al.* 1978, Lang 1967, Sarason *et al.* 1991, Schaffer *et al.* 1992, Valerio and Dalgard 1975).

In a study to evaluate semen from capuchins monkeys it was found that the most common abnormalities were detached heads and bent and coiled tails (Bush *et al.* 1975). Although no correlation with motility could be demonstrated, it appeared that abnormalities were most commonly found in dead spermatozoa as determined by supra-vital staining. Tail defects were again the most common abnormality in marmoset spermatozoa with a median of 50% (Cui *et al.* 1991). Head defects were on the other hand rare with a median of 4.5%. The same applied to tamarins where the most frequent abnormalities were midpiece defects (Harrison and Wolf 1985). Coiled tails, cytoplasmic droplets and detached heads were also observed but in very small numbers. The overall rate of defective spermatozoa was low and more than 5% was considered abnormal for this species.

Abnormal forms were found to be also rare in the spermatozoa of rhesus monkeys and none of the ejaculates of 100 males investigated contained more than 5% abnormal forms (Harrison 1980). The most common defect found was abnormal midpieces. Others were amorphous heads, cytoplasmic droplets, small heads, detached heads and coiled tails but none of these occurred more frequently than 2-3%. Abnormal spermatozoa were relatively rare in cynomolgus monkeys and less than 10% of all ejaculates contained more than 30% abnormal forms including 15% bent and 8% kinked tails (Mohamed *et al.* 1987). Only tail abnormalities appear to have been observed during this study.

The trend towards the prevalence of tail defects was also observed in Sulawesi macaque sperm (Thomson *et al.* 1992). Coiled, kinked and short tails were the main defects found in four ejaculates from four males. Only few head defects occurred in three individuals but the type of defects were not reported. The overall rate of morphologically normal sperm was high (96.8%).

Ejaculated spermatozoa from cynomolgus monkeys were morphologically similar to those aspirated from various regions of the epididymis except for the location and occurrence of the cytoplasmic droplet (Mahony *et al.* 1993). The ultrastructure of bonnet monkey sperm was similar to that of other non-human primate species (Kalla *et al.* 1986).

It appears that in most non-human primate species the most common abnormalities are confined to the tail while head defects are rare.