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Study about the normal semen quality of free-ranging Southern White Rhinoceros located in South Africa

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Abstract

Various aspects are challenging the population of the Rhinoceros, such as poaching for their horn and their homogenous gene pool having the biggest influence on their numbers. The further conservation of this species may require intervention by breeding management and various assisted reproductive technologies (ART) to improve their population numbers.

Ejaculates were collected by electroejaculation in 7 male rhinoceros. Basic semen analysis techniques were used to evaluate the semen in field conditions including macroscopic evaluations to microscopic evaluations to evaluate individual sperm cells.

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The average ejaculate per rhinoceros during this study was 53.75±47.95ml with an average 3.68 ± 3.16ml ejaculate per stimulation. The ejaculate had a predominant milky consistency with a white colour. The mass motility of the 7 rhinoceros were 3.5, with the progressive motility of the sperm being 69%. The sperm had a 70% normal morphology.

All the parameters that were evaluated; the extensive free-ranging group had better semen quality except for normal semen morphology which was similar in both groups.

Normal semen qualities of free-ranging Southern White Rhinoceros are 3.68±3.16ml, white milky with mass motility of 69% and progressive motility of 70%.

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1. Introduction

The Southern White Rhinoceros (*Ceratotherium simum simum*)(SWR) is a species facing numerous conservation challenges, including habitat loss and poaching. Despite extensive research on various aspects of rhinoceros conservation, a notable gap exists in understanding the normal semen quality of free-ranging populations that may be used in assisted reproduction technologies (ART) to aid conservation efforts. Semen quality plays a vital role in reproductive success, and investigating this aspect in the SWR contributes valuable insights into their overall reproductive health. While studies on captive rhinoceros populations have contributed to our understanding of reproductive physiology[1], applying these findings to free-ranging individuals poses unique challenges due to environmental differences and natural behaviours. Therefore, an investigation into the normal semen quality of free-ranging southern white rhinoceros is essential for a general understanding of their reproductive biology to achieve reproductive success.

This paper presents research that aims to fill the knowledge gap by employing modern semen analysis techniques [2] to assess sperm parameters in a natural, uncontrolled environment. Through this approach, we aim to establish the baseline semen quality of free-ranging southern white rhinoceros and identify potential factors impacting reproductive success. This knowledge will be instrumental in guiding conservation strategies and enhancing the overall management of this endangered species.

2. Literature Review

2.1 White Rhinoceros Population in South Africa

During the early 1900s, the white rhinoceros numbers in Natal (today known as the KwaZulu-Natal province), in the Umfolozi/Hluhluwe reserve in Northern Zululand, were reduced to below a hundred individuals [3]. The accessibility of rhinoceros, their desirable meat, the use of their skin for whips, and the lucrative trade in their horns overseas all contributed to the decline in the population. The presence of the Tsetse fly (*Glossina*), carrying the deadly Nagana disease, played a crucial role in inadvertently protecting the remaining population, as hunters avoided areas where the flies were prevalent. The former Natal Parks board intervened, successfully conserving the remaining rhinoceros by relocating them to government and privately-owned game reserves [3]. Natal Parks, Game and Fish preservation board was an organization established in 1947 to enforce wildlife laws in the province of Natal; the name was later shortened to the Natal parks board.[4]

By 2012, the SWR population had grown to a population size of 21 316 individuals. However, after this, poaching started to increase, leading to a 15% decrease in the number by the end of 2017; reducing the population size to 18 064 individuals [5]. In 2021, the number further reduced to only 15 942 individuals. Following the declining population trend, recent data shows that the amount of poaching which occurred in 2022 (561 poached rhinoceroses) were increased compared to the previous year of 2021 with 451 poached individuals.[6]

Despite the alarming poaching rates, research by the IUCN, indicates that South Africa is still the stronghold of the Southern White Rhinoceros (SWR) population, supplemented by reintroductions into Botswana, Eswatini, Mozambique, Namibia, and Zimbabwe. Presently, the species occupies 422 distinct locations across its range.[7]

In 2023, the population of SWR has increased for the first time since 2012. The SWR number has increased to 16 803, an increase of 5.6% [6].

2.2 Gene diversity

At the turn of the 19th century, only one SWR population remained in South Africa [3]. Research conducted by Sánchez-Barreiro et al. (2021) indicates a significant loss of genomic

diversity in the SWR population. Their findings revealed evidence of a founder effect originating from the remaining population in the Umfolozi/Hluhluwe reserve in Northern Zululand. Founder effect refers to a reduction in genetic variation that occurs when a small group of individuals establishes a new population.

Their analysis highlighted an apparent affinity between the genetically homogeneous modern SWR population and historical samples obtained from Natal. This suggests that the genetic composition of present-day SWR populations can be traced back to the founding population in the Umfolozi/Hluhluwe reserve.

Nowadays, wildlife parks and private rhinoceros owners try to prevent the negative effects of inbreeding by manipulating the paternal line through translocation and artificial insemination.

2.3 Reproduction

White rhinoceros births have been documented every month of the year, indicating that SWR reproduction is not restricted to seasonality [8]. Young southern white rhinoceros females typically undergo their first oestrous at about five years of age. They remain in a sub-adult group until their first calve is born at age 6.5-7 years.[9]

The gestation period of a white rhinoceros is 16 months. Oestrus can reoccur in 30-day intervals if the cow is not fertilized. There is a notable increase in mating activity from October to December, which follows the onset of spring rains and a subsequent peak in calving from March to May [8]. The inter-calving period can vary between 2 and 3 years.

According to a study done by Patton et al. on the reproductive cycle length of white rhinoceros, females have a cycle length of 35.4 ±2.2 days, with 59% of their study group falling between 29 and 41 days.[10]

Young males are only considered adults when they become solitary between 10 and 12 years of age, coinciding with the time they establish their dominance [9].

Male Anatomy

The musculocaverous penis is completely covered by the preputial fold in the relaxed stage. As the penis transitions to a semi-erect state, it curves caudally upon release from the sheath. During complete erection, the penis straightens, causing the tip to swing forward [11]. The male rhinoceros possesses distinctive lateral penile flaps that, during intermission, would be

located inside the vagina. The flaps significantly increase the diameter of the erect penis [12]. Their testicles, like that of stallions, are positioned horizontally.

As seen in diagram 1, the male rhinoceros features vesicular gland sacculation resembling those of bulls, a triangular prostate similar to that of stallions, and lack an ampulla characteristic of boars [12].

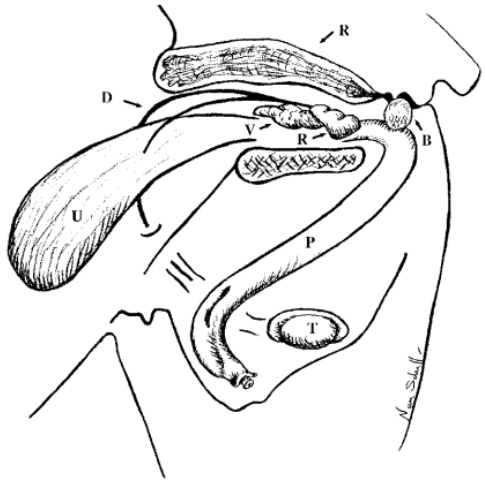

Diagram 1: Abdominal orientation of the male rhinoceros reproductive tract P= penis; T=testis; D=ductus deferens; B=bulbourethral gland; A=prostate; V=vesicular gland; U=urinary bladder; R=rectum [11]

The testes and closely attached epididymis are located within the dorsal region of the preputial fold. As the penis relaxes, the placement within the preputial fold changes from a vertical orientation, which is easily observable and palpable, to a more horizontal orientation adjacent to the inguinal rings, making it less palpable [13, 14].

The presence of thick skin, a dense testicular capsule, and the changing position restricts the possibility of drawing definitive conclusions regarding the functional status through testicular palpation. Clinical assessment of the testes and the accessory sex glands, transcutaneous ultrasound of the testes, and transrectal ultrasound of the accessory sex glands are required to evaluate the fertility. Animal restraint, either in a chute or through sedation, is necessary for a thorough examination.

Sperm Morphology

Semen and sperm quality related to sperm function and fertility

Semen is a liquid suspension that contains spermatozoa, male gametes, and secessions from the accessory organs of the male reproductive tract. Seminal plasma is the liquid portion of semen that is formed at ejaculation [15, 16].

Sperm cell

Spermatozoa are formed in the seminiferous tubules of the testes. These tubules contain developing cells that would finally develop into the male gametes. Fully formed spermatozoa contain a flattened head containing the nucleus and a tail that aid in cell motility. A plasma membrane covers the entire spermatozoa. As seen on Diagram 2, the acrosome is between the plasma membrane and the anterior portion of the sperm head. A neck connects the sperm head to the tail.

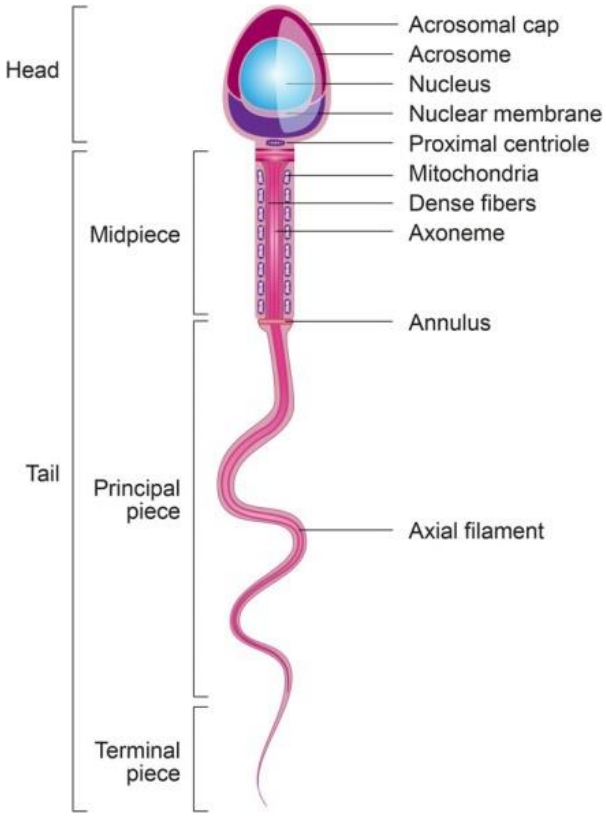

Diagram 2: A diagram depicting the different parts of a normal sperm cell [17].

Sperm head

The head is oval and flattened and contains compact chromatin. Chromatin is made of deoxyribonucleic acid (DNA). The content of the nucleus is haploid since it originates from meiotic cell division that takes place during sperm formation [15, 16].

Acrosome

The acrosome is located at the anterior end of the sperm nucleus. A thin double-layered membranous sac layered over the nucleus at the last stage of sperm formation. This structure

contains acrosin hyaluronidase and other hydrolytic enzymes that aid in the fertilization process [15].

Sperm tail

The tail of the male gamete consists of the neck, middle piece, principal piece, and end piece. The neck, or connecting piece, features a basal plate that fits into a recess posteriorly of the nucleus. This basal plate continues posteriorly with nine outer coarse fibers that extend along most of the tail [15, 16].

The section of the tail between the neck and the annulus is called the middle piece. The central core of the middle piece, along with the entire tail, forms the axoneme, which is made up of nine pairs of microtubules arranged radially around two central filaments. In the middle piece, this 9 + 2 arrangement is surrounded by the nine outer coarse or dense fibres associated with the axoneme's doublets. Numerous mitochondria encase the axoneme and dense fibers of the middle piece, arranged in a helical pattern along the tail's longitudinal fibres, providing the energy necessary for sperm motility [16].

The principal piece extends posteriorly from the annulus to near the tail's end, consisting centrally of the axoneme and its associated coarse fibres. A fibrous sheath offers stability to the tail's contractile components.

The end piece, located just beyond the fibrous sheath's termination, contains only the central axoneme covered by the plasma membrane, which is vital for sperm motility. The outer pairs of microtubules in the 9 + 2 configuration create bending waves in the tail through sliding movements between adjacent pairs.

The protoplasmic or cytoplasmic droplet, typically detached from ejaculated spermatozoa, consists of residual cytoplasm. While it is generally viewed as abnormal for ejaculated sperm in most species, the droplet can be retained in the neck region as a proximal droplet or near the annulus as a distal droplet [15, 16].

2.4 Semen Collection and Evaluation

Semen collection has become a regular practice to evaluate the fertility of a male animal as well as in the use of assisted reproduction procedures. The management of threatened species has become prioritized; thus, the successful collection of semen from these species has become more important. Various semen collection techniques have been developed for domestic animals but not for the rhinoceros species. These techniques have been altered to comply with the needs of rhinoceros semen collection. One technique is electroejaculation on an anesthetized rhinoceros [18], and another is semen collection by using an artificial vagina (AV) which is mostly unsuccessful because the penis cannot get fully erect in the AV as it has lateral folds [11, 12]. Another factor that contributed to the unsuccessful use of AV was the fact that the animals needed to be preconditioned by teasing the male before semen collection [11].

Electroejaculation is the most common technique to collect semen in rhinoceros [13, 19]. The handheld electronic probe needed to be altered since the size of the rectum, as well as the position of the accessory sex glands, are located differently than domestic animals [20]. Electroejaculation is done under general anaesthesia in untrained, captive, or wild rhinoceroses [18].

Constant sperm production throughout the year became evident by collecting good quality sperm by electroejaculator in White Rhinoceros [1].

While the database regarding ejaculate characteristics and sperm quality for rhinoceros continues to grow, it must be evaluated with caution since there are significant variations in sample quality observed between collection attempts, even within the same animal [18].

Evaluation

Semen is composed of two primary components: Sperm and Seminal fluid. Sperm is produced in the seminiferous tubules of the testes, and seminal fluid is produced in the accessory sex gland. Semen analysis examines both these components [21].

Spermatozoa of the rhinoceros are of similar size to those of stallion spermatozoa [22].

During semen analysis, the macroscopic and microscopic characteristics of semen are evaluated. Macroscopic analysis involves volume, viscosity, colour, liquefaction, and pH. Microscopic analysis is the number of sperm, motility, morphology, vitality, and presence of leucocytes [23]. Computer-assisted sperm analysers (CASA) are associated with a better

method for assessing the quality of fresh semen. It is measured faster, as well as multiple parameters of semen, accurately and precisely [24].

Semen volume plays a crucial role in semen evaluation as it, in combination with the concentration, helps determine the total amount of sperm ejaculated [25]. The volume is greatly influenced by the semen collection technique used [18], and factors such as the animal's age and frequency of collections also affect volume [26].

Similar to the volume, measuring sperm concentration would help us to determine the total sperm number of the ejaculate [25] The concentration per mL of semen can be determined by using a hemocytometer, a microcell, or a photometer. Using these methods there could be possibilities of over or underestimation of the sperm concentration [27]. Computer-Assisted Sperm Analysis (CASA) presents a potentially better alternative for determining sperm concentration [28].

Morphology of sperm is one of the most important indicators of fertility. It is collectively accepted that sperm with a normal morphology has an important effect on fertility both in vivo and in vitro [29, 30]. Abnormal types of sperm are classified based on morphology, associated with defects in the head, neck, midpiece, and default queue [31]. Various fixation and smear preparation techniques are being used to evaluate sperm morphology. Automated Sperm Morphometry Analysis (ASMA) refers to the computerized analysis of sperm morphology, enabling the assessment of live sperm [32]. ASMA efficiently distinguishes between normal and abnormal sperm, addressing technical variations and providing estimations with improved accuracy and precision in sperm morphology assay. The application of staining enhances the elaboration of sperm morphology.

Advancements in sperm motility assessment involve using light and phase-contrast microscopes, typically with 20x and 40x objective lenses, which have been shown to produce reliable results [33]. It is recommended that a microscope with a stage warmer that is adjustable to 37°C, with sufficient magnification, be used to visualize the sperm [25]. It is not advised to use a light microscope due to problems with visualizing immotile sperm, especially at low magnification, which could lead to false high-motility results [2].

Ejaculate colour and viscosity

Normal semen colour ranges from white, ivory and yellow colour [14, 16]. There is a lot of factors that contribute to the colour of semen. Mainly, the concentration of sperm and their diet can influence the colour of the ejaculate. Greyish white is an indication of lower sperm concentration compared to ivory or cream, which could indicate a higher concentration of sperm [15, 34].

Abnormal colours of semen may include yellow, which is an indication of urine contamination; green, a colour indicating pus presence; red or brown, contamination of blood; red being fresh blood; and brown, being older. All of these contaminants can influence the semen quality [35].

Semen viscosity and consistency are determined by the concentration of sperm. The consistency of semen can range from watery, milky, or creamy. A more watery consistency may indicate lower concentrations of sperm present in the ejaculate[36].

Volume

Volume is an important part of semen evaluation since it indicates if a representative sample of semen has been collected. However, the volume is largely influenced by the collection process, thus making volume not a relevant measurement. The volume can be influenced by several factors, including the age of the animals, collection methods, and the frequency and the season of the year [36, 37].

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Sperm motility

For sexual reproduction to take place, the unification of male and female gametes is required. In mammals, the most remarkable event in the fertilization process is the journey, traveling thousands of times their body length through the confined oviducts to the egg that occurs through the active swimming behaviour of the specialized male gamete [38].

3. Materials and Methods

3.1 Source of biomaterial samples

The topic of this thesis arose when I contacted Dr. Morne de la Ray, the Co-owner of Embryo Plus. Embryo Plus does significant work in developing and furthering research in animal genetics and plays a major role in the conservation of animal genetics. The data that Embryo Plus collected from 2016 to 2021 was made available for this author's use in this paper. This set of data presents significant insight into the conservation efforts, via reproductive techniques, of Southern White Rhinoceros in South Africa.

The samples used in this study are semen samples taken from 7 Southern White rhinoceroses from the South African provinces of Limpopo and Mpumalanga. Due to security risks, the exact locations of the rhinoceros cannot be disclosed. The rhinoceros evaluated in this study are divided into extensive and semi-intensive systems. In the extensive system, the animals were kept and grazed on natural veld, while in the semi-intensive system, the animals were provided shelter and had access to natural veld.

The semen samples were collected from November 2016 through to October 2021.

3.2 Anaesthesia

All semen sampling procedures occurred when the animal was under general anaesthesia. The bulls were identified based on their specific features or ear notches. If microchips were present in the animals, after anaesthesia the chip could be read by a microchip-reader.

The drug dosage was based on an on the estimation of the animal's body weight by experienced personnel. The anaesthesia dose was delivered via a 3ml plastic dart by using a dart gun from a helicopter or from the back of a pick-up. The dart sites include the shoulder, neck, or low hindquarter of the rhinoceros.

All darting and drug administration were completed by qualified and experienced veterinarians. Other experienced staff of the team frequently monitored the general well-being, breathing and heart rate of the animal during the sampling procedures.

The anaesthesia combinations used were based on prescribed drugs and amounts in the book "Chemical and Physical Restraint of African Wild Animals" [39]. Anaesthesia was achieved by using 150mg Butorphanol, 14mg Medetomidine, and 10mg Midazolam for induction. Anaesthesia was maintained with a constant rate infusion with Guaifenesin, Ketamine and Medetomidine. Prostaglandin was then injected to induce the contraction of the vas deferens to increase the volume of ejaculate.

3.3 Biomaterial Collection Techniques

Semen collection techniques

During the collection procedure, the bulls were kept in either standing or lateral recumbency as seen in Image 1b, while subjected to the electrostimulation protocol described in rhinoceroses [21]. Faeces were removed from the rectum before the custom-designed probe was inserted into the rectum. The special electroejaculater (Image 1a) that was used was designed by Embryo Plus for the successful ejaculation of the rhinoceros bulls. The device was specifically designed to stimulate the accessory sex glands. Before electrical stimulation, the prostate and seminal vesicles were stimulated manually. The electrostimulation procedure consisted of sequences of stimulation patterns consisting of five to eight electrical stimulations based on the principle of rest and stimulation. The El Toro 3 was used as an electro-ejaculator power source, with a voltage ranging from 2.3 to 9.5V. Stimulation started with a low voltage and proceeds with an increase in voltage with each stimulation. The stimulation patterns were adapted to the response of the bull; variations can happen between individuals. The erect penis was then manipulated into a silicone vagina, which was connected to a 15ml tube. The collection tubes were insulated to protect them from direct sunlight and adverse temperature deviations. Over-stimulation of the urethra may result in urination. Urination is signalled by strong pulsation of the penis and urine spraying [1].

The collection tubes were replaced regularly to prevent the semen sample from being contaminated with urine which could kill the sperm cells [40]. A series of ejaculation samples were collected for each rhinoceros bull. After each sample, a semen analysis was performed to evaluate the concentration of sperm. Samples were collected until the ejaculate became watery which indicated a lower concentration of sperm present. The number of ejaculate samples in the series for each bull was variable between the rhinoceroses.

Directly after the ejaculate sample was obtained, the semen was evaluated in a shaded workstation. The macroscopic and microscopic characteristics of the semen were analysed. The macroscopic assessment involved the evaluation of the volume, colour (grey, white, ivory, colourless or yellow), and consistency (water, thin milk, milk, thin cream, cream, thick cream).

The microscopic assessment was performed on microscopic slides, pre-warmed on a heat stage at 37°C. Microscopic evaluation involved the analysis of mass motility and progressive motility. The evaluation was performed by using a light microscope.

As soon as the sample arrived at the field workstation, it was labelled accordingly. All the glass slides as well as vials were labelled with the animals' names or identification (ID) number and the date of the collection.

Image 1a: Probe specifically built for rhinoceros stimulation by Embryo Plus

Image 1b: A rhinoceros under general anaesthesia in lateral recumbency

3.4 Macroscopic semen evaluation

Semen colour and consistency

The appearance of each semen sample was evaluated. Semen samples were classified as ivory, white, grey, colourless, or yellow. Ivory indicates a highly concentrated semen, and white colouring indicates a somewhat lower sperm concentration. The grey discoloration of semen may indicate low sperm concentration and colourless indicating very low or absent sperm.

The consistency of semen normally thickens with the increase in concentration of sperm or, abnormally, by the presence of viscous substances such as mucus or pus in the semen.

Consistencies were described as water, thin milk, milk, thin cream, cream, and thick cream.

Semen volume

The semen sample fractions were measured in a 15ml collection tube which had measurement indicators on it. The final volume for each sample was recorded.

3.5 Microscopic semen evaluation

All the disposables, such as slides, cover slips, and pipettes, were pre-heated to 37°C. The light microscope has a x10 eyepiece and an objective magnification of x10 and x40.

The lens of x10 magnification is used for progressive motility, and the x40 magnification is used for morphological examination by evaluating two groups of 100 spermatozoa in random areas of the smear. Mass motility was evaluated using a .x4 objective lens.

Semen mass motility

A small sample was taken from the diluted semen sample with a Pasteur pipette, placed on a pre-heated glass slide, and placed on the heated microscope table. The mass motility was evaluated, and a number 0-5 was allocated. Zero indicates no movement of sperm and five indicates strong waves, reaching the edge of the droplet, forming whiplash effects at the edges.

Sperm progressive motility

For progressive motility, the slide is used with a coverslip placed on top of a drop of semen. Using a 10x magnification, the progressive motility could be determined. Under the microscope, the individual sperm cells were visualized, and a ratio was concluded between

motile and immotile sperm. For example, a 6:1 ratio indicated six motile sperm and one immotile sperm, thus concluding a 85% progressive motility.

Sperm morphology

A drop of semen was used to make a smear on a microscope slide to evaluate the sperm morphology. After drying, the slide was prepared using the Spermac Stain Kit. Two groups of 100 spermatozoa in random areas of the smear was then examined under a light microscope at 40x magnification to determine the percentage of normal spermatozoa present by examining the shape and structure. A schematic drawing as seen in Diagram 3 was used as reference[41]. An average was then calculated to give a percentage of normal spermatozoa and spermatozoa with major and minor defects. Major defects are acrosome, head, pyriform, major midpiece, proximal droplet, and dag defects. Minor defects include loose heads, base defects, minor midpiece defects, distal droplet and tail defects.

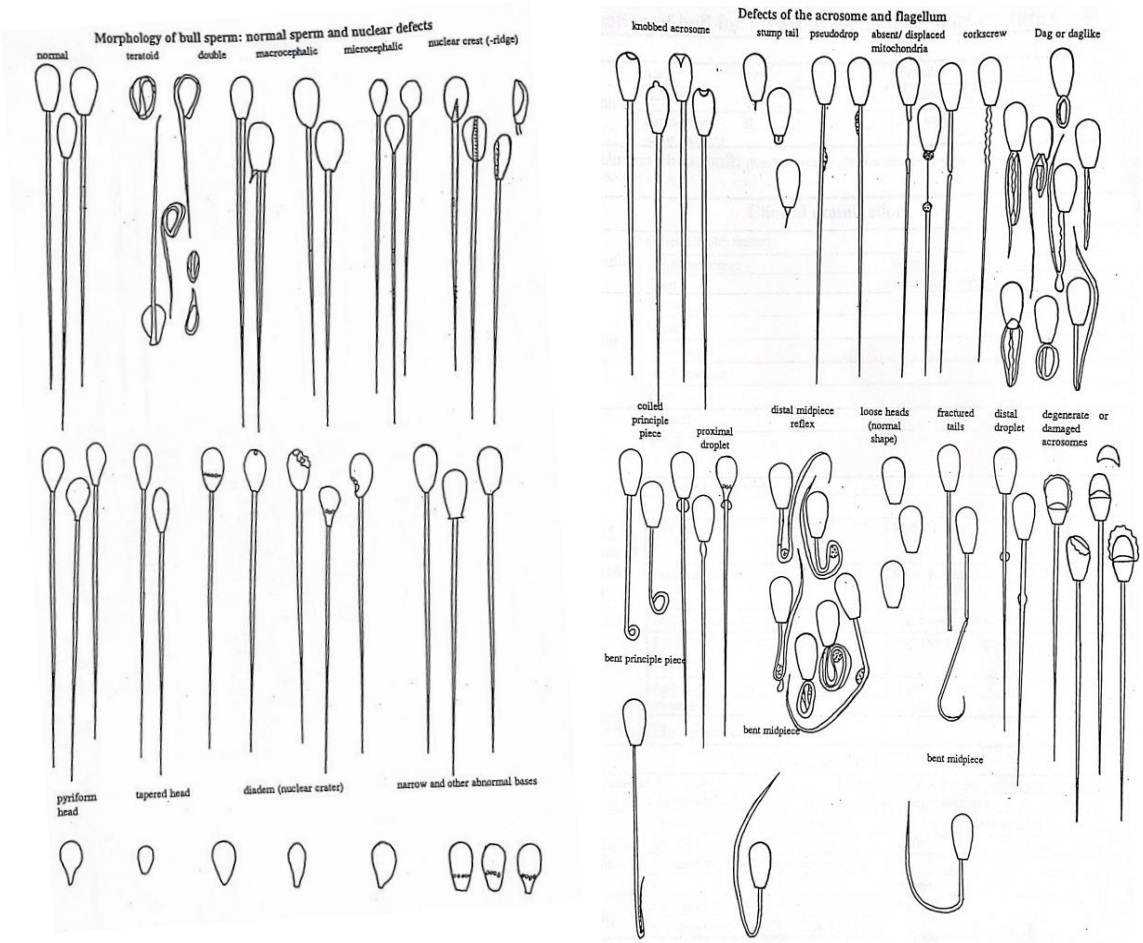


Diagram 3: Schematic Drawing used as a reference to classify sperm morphological abnormalities during the Spermac Stain kit semen smear evaluation [40]

4. Results

Basic semen evaluation techniques were performed on 98 ejaculates that was collected from 7 adult rhinoceros bulls on 8 occasions. Four rhinoceros belonged to the extensive group and 3 belonged to the Semi-intensive group. The semen samples were collected between November 2016 and October 2021. Summarized results of collected semen samples can be seen in Table 1 below.

Table 1: Results of the 7 rhinoceros ejaculate that were evaluated

No	Date	Location of collection	No. of collections	Total volume	Mass motility	Progressive motility(%)
Rhinoceros 1	21/11/2019,11/03/2020	Limpopo	16	34.5	2	49
Rhinoceros 2	10/06/2021	Limpopo	13	57.5	1	80
Rhinoceros 3	06/10/2021	Limpopo	15	58	1	N/A
Rhinoceros 4	28/01/2020	Mpumalanga	1	6.5	3	80
Rhinoceros 5	28/01/2020	Mpumalanga	10	21	2	60
Rhinoceros 6	19/02/2020	Limpopo	18	37	2	70
Rhinoceros 7	22/07/2020	Limpopo	24	142.5	2	60

4.1 Macroscopic results

Ejaculate volume

The average ejaculate volume per stimulation measured during this study was 3.68 ± 3.16ml. The recorded ejaculate volumes ranged from 0.5-17.5ml

Table 2: Ejaculate volume analysis of all ejaculated evaluated

Parameter	N	mean	Std dev	median
Volume(ml)	96	3.65	3.18	2.5

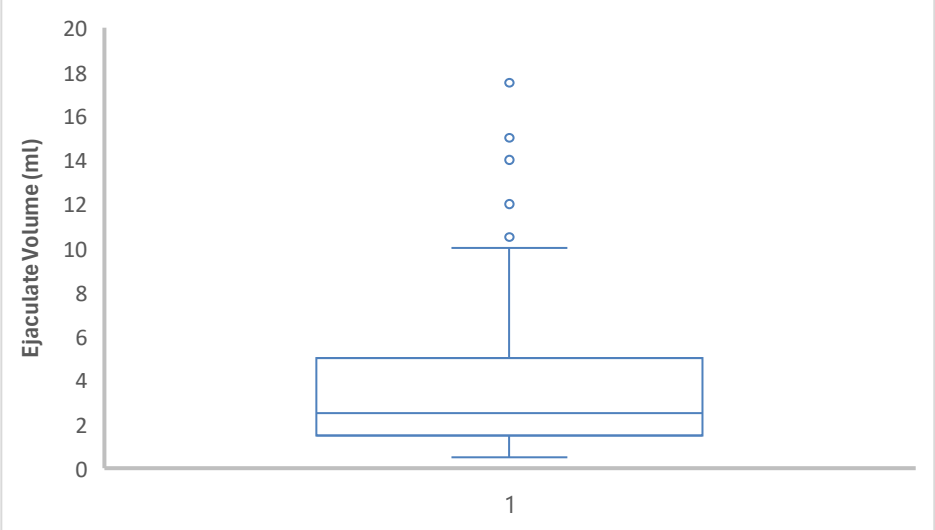


Figure 1a: Box and whisker figure illustrating the individual ejaculate volume of semen

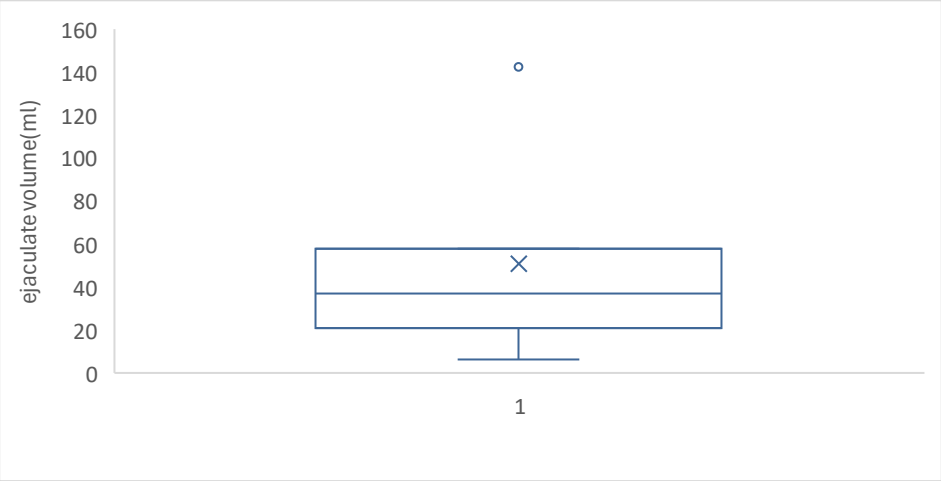


Figure 1b: Box and whisker figure illustrating the total volume of semen

As seen in Table 3 the average total volume of ejaculate during this study was 53.75 ± 47.95 The total ejaculate volumes ranged from 6.5-142.5ml. The semi-intensive group had a slightly higher total volume compared to the Extensive group.

Table 3: The total volume analysis of each rhinoceros collective ejaculate volume

Parameter	N	Mean	Std dev	Median
Volume(ml)	7	53,75	47,95	37

Consistency and colour

The consistency of the semen varied considerably among different rhinoceros individuals as well as within the same rhinoceros with consecutive stimulations. As seen in Figure 2, the range was between thick milk in some cases and poor water consistency in others. The colour of the semen samples differs between and within one rhinoceros with different stimulations. Colour ranged from a good ivory colour to a less favourable grey colour as seen in Figure 3. Any contamination of the sample with urine or any other contaminant had been discarded and was not analysed in the study.

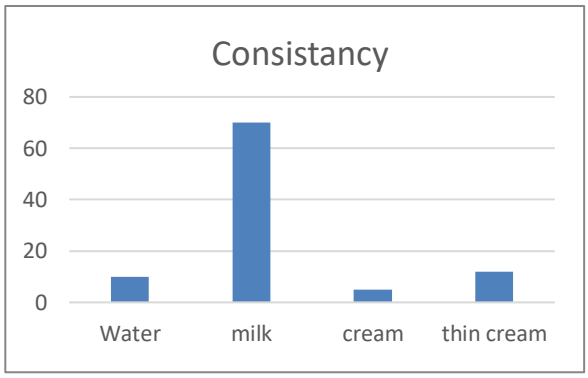


Figure 2: Semen consistency of all semen collected

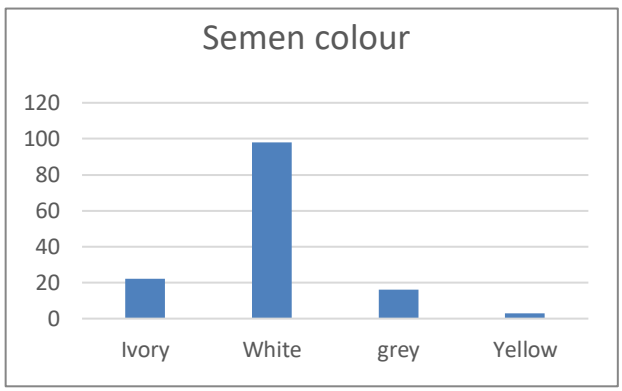


Figure 3: Semen colour of all rhino semen collected

4.2 Microscopic

Mass motility

The mass motility of these rhinoceroses ranged from 0, with no mass motility, to 3.5 which has a moderate mass motility.

Progressive motility

As expected, there were also big variations between individuals as well as within the same rhinoceros's progressive motility. The progressive motility ranged from a very low 5% to a staggering 90% progressive motility.

Morphology

The sperm cell of a Southern White Rhinoceros is comprised of a head and a tail. The longest part, which is the tail includes a midpiece, principal piece and an end piece.

The normal morphology of the rhinoceros sperm ranged from 42% normal morphology to 87% normal morphology.

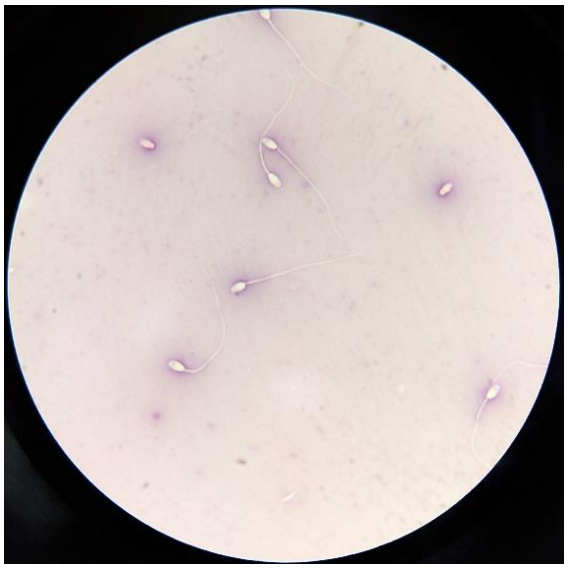


Image 2: Sperm under a microscope. two loose heads and 6 normal sperm cells

5. Discussion

This study was aimed to characterize the semen and sperm parameters of ejaculates collected from free-ranging Southern White Rhinoceros populations in South Africa. An approach was employed to analyse the sample, which included routine macroscopic and microscopic semen analysis techniques. The results presented are based on 7 adult rhinoceros which were evaluated.

5.1 Macroscopic semen evolution of Southern White Rhinoceros

Colour and Viscosity

Samples were collected from 7 healthy adult male rhinoceroses between 2016 and 2021. The collected ejaculate samples generally had a white colour with minor variation in its consistency, which was mostly milky. Not all the samples that were collected had spermatozoa, and some samples were contaminated with urine, which was not evaluated and was discarded. It is widely accepted that electroejaculation can influence the consistency of the semen sample produced and those results obtained typically are dependent on the technique used. The multiple male accessory sex glands such as the Bulbourethral, prostate, and vesicular glands, could be responsible for the consistency of the semen [12]. However, in this study, a lower consistency was observed in the rhinoceroses' semen. During the stimulation and collection process, it could only be speculated which of the fractions could be sperm-rich or just glandular fluid before it was confirmed under the microscope. Thus, the collection tubes were replaced after each stimulation to prevent contamination with urine, which would result in the discard of the sample.

The colour and consistency of the semen differed marginally between the extensive and semi-intensive groups. The extensive group had a more favourable colour of semen such as Ivory/white, whereas the intensive group had mostly a white colour to the ejaculate samples. The consistency for the extensive group was also more favourable with the majority being milk, cream and thin cream in comparison with the semi-intensive group that mostly had Milk/water consistency. Based on these findings, it may be concluded that the extensive group had better semen quality based on colour and consistency.

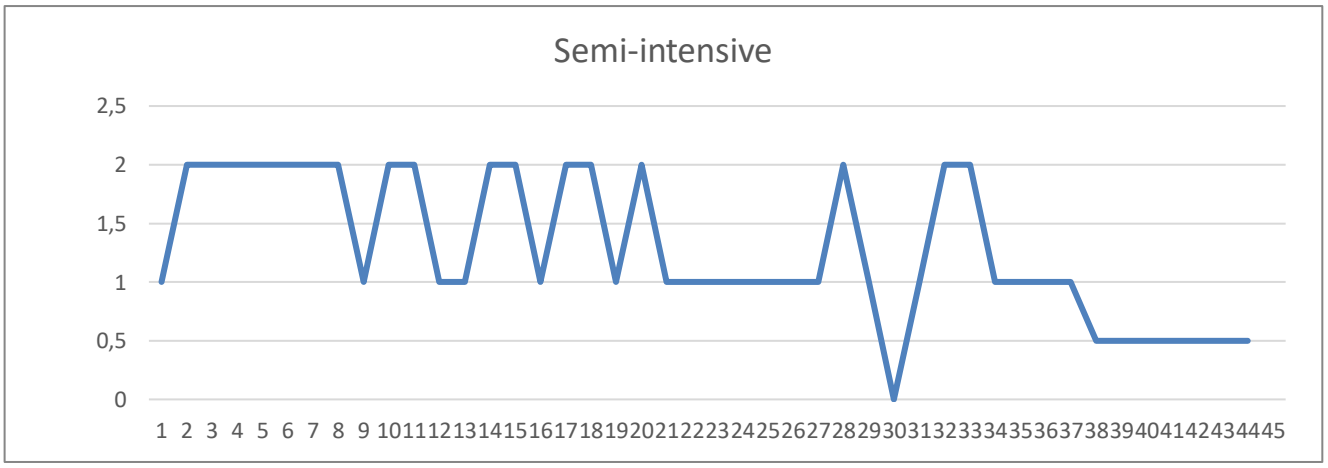
Ejaculate Volume

The average recorded rhinoceros ejaculate that was collected by electro-ejaculation in this study was 53.75 ± 47.95ml (range: 6.5-142.5ml). This is much higher than what was described by other articles [1, 42]. Unfortunately, we do not know how it relates to natural ejaculation since training individuals to ejaculate into an Artificial vagina has been unsuccessful [11, 18]. Further studies and experiments should be done to find a more repeatable way of collecting data to compare rhinoceros semen parameters. Artificial insemination and in-vitro fertilization (IVF) need high-quality semen that needs to be collected to either be used fresh or frozen.

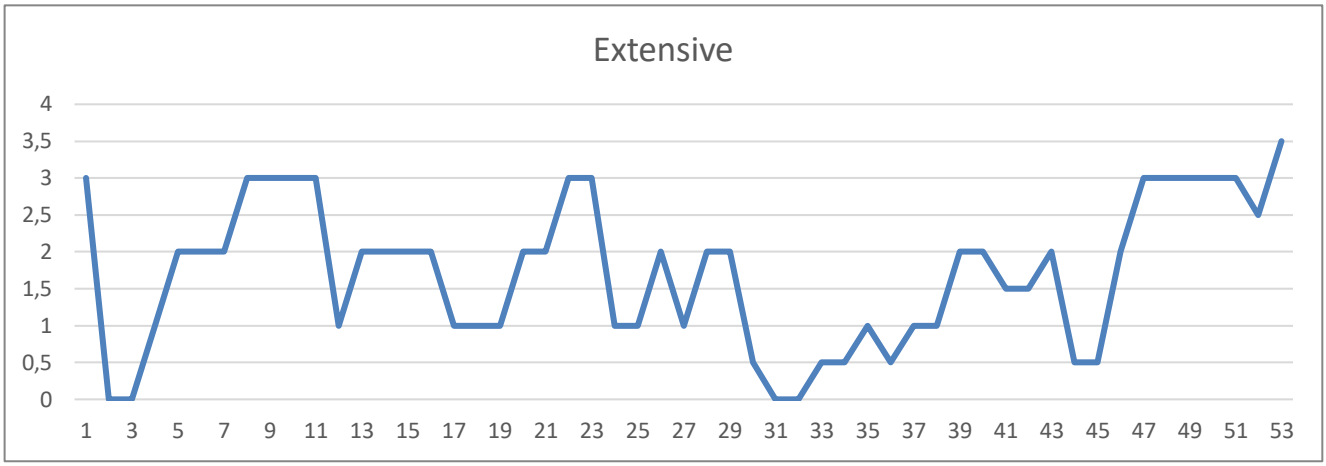
5.2 Microscopic semen evaluation of the southern white rhinoceros.

Mass motility

As seen in Graph 1, it was observed in this study that the 7 rhinoceroses did not have a very high mass motility compared to those of farm animals like horses, swine and cattle. The mass motility, which evaluated out of 5, was for the semi-intensive group an average of 1.3 ± 0.6 with most having a 1 or 2 mass motility.



Graph 1: Mass motilty of the semi-intesive rhinoceros group

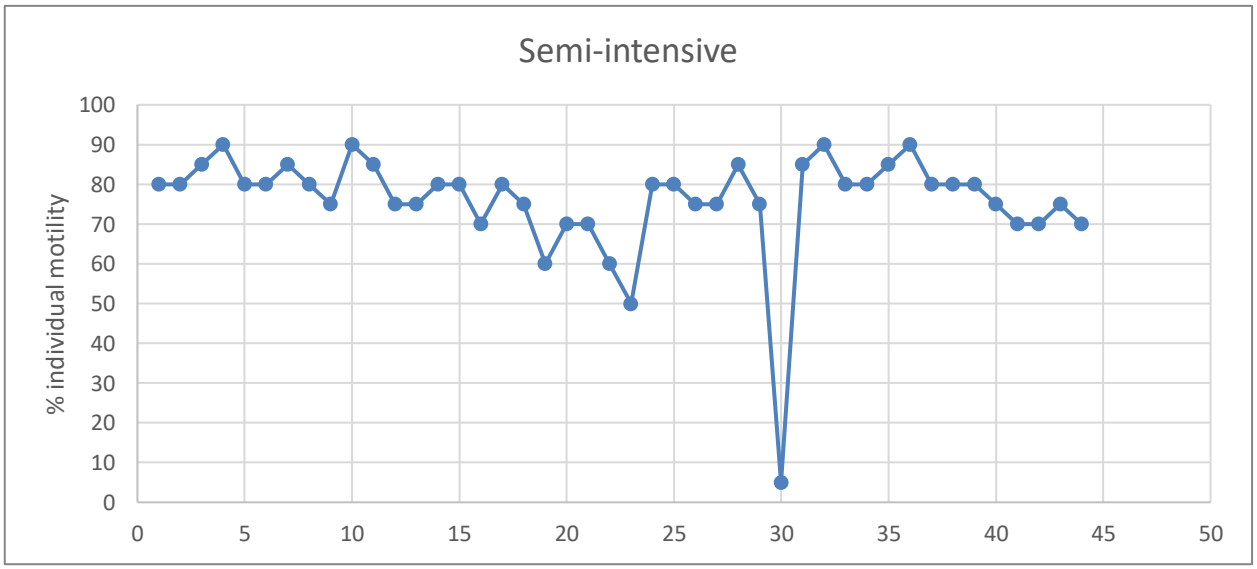


Graph 2: Mass motility of the extensive rhinoceros group

The mass motility of the extensive rhinoceros was better than that of the semi-intensive group. An average grading of 2.1 ± 0.9 was given out of 5 for the extensive group, with the lowest being 0 and the highest 3.5. Most had a 2 or 3 grading for mass motility as seen in Graph 2.

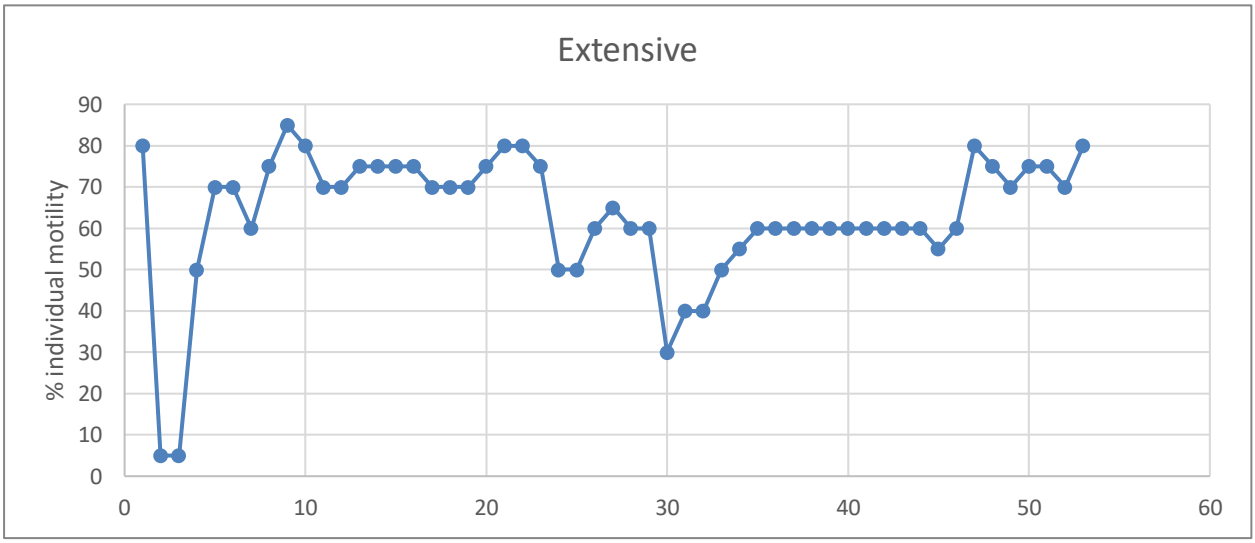
Progressive motility

The progressive motility, as seen in Graph 3, of the sperm cells of the rhinoceroses in this study did not vary exceptionally between each other. The average progressive motility for the semi-intensive group was 62% which had progressive forward motion, with 5% being the lowest and 90% being the highest in progressive motility in this group.



Graph 3: Progressive molitility of Semi-intensive rhinoceros group

Graph 4 depicts the progressive motility of the sperm cells of the rhinoceroses in the extensive group. Extensive group had an average motility of 67% which had progressive forward motility, with 5% being the lowest and 85% being the highest progressive motility in this group.



Graph 4: Progressive motility of the extensive rhinoceros group

The combined average progressive motility of both rhinoceros groups that were evaluated in this study was 65% progressive forward motility.

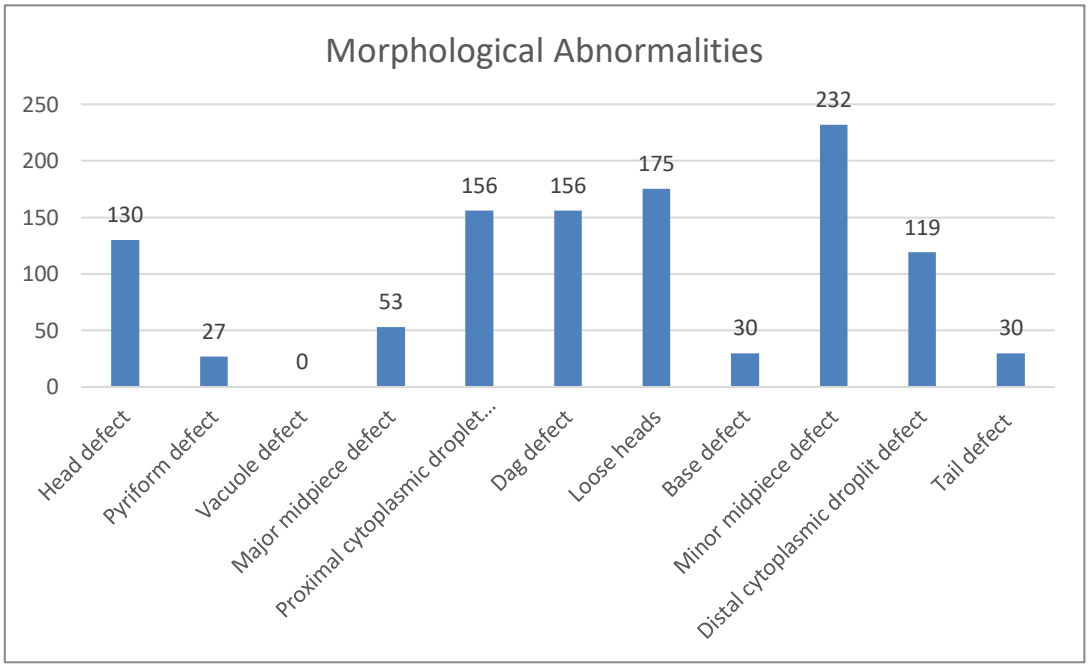
Besides the progressive motility being better in the extensive group, a higher volume of sperm, better colour, and consistency were also noted. This could be caused by several unknown factors. One known factor that could be considered is that rhinoceros live in small family groups that consist of sub-adults, cows, and calves. The dominant males are generally more solitary and very protective of their territory [3]. In the semi-intensive system, the rhinoceros bulls are collected in a sheltered boma causing challenging hierarchical dynamics, resulting in the consumption of sub-optimal feed and nutrition. This can be the cause of the lower semen quality of the semi-intensive group. On the other hand, the extensive group had free access to feed and had no hierarchical dynamic interference, which could help the rhinoceros optimize their condition and thus have a positive effect on their semen quality. Unfortunately, there is not a lot of information about the influence of nutrition on the semen production of Southern White Rhinoceros.

The differences between the 2 groups could also be due to the sample size being so limited. The influence of a single rhinoceros could have been too great and therefore could have led the study in a certain direction, which gives false results.

Morphology

The rhinoceros ejaculates analysed during this study contained an average of 70 ± 11% morphologically normal spermatozoa. As seen in Graph 5, the abnormal morphologies were common across all ejaculate samples analysed in this study. No difference in sperm morphology was recorded between the two groups in this study nor in the number of normal morphologies recorded.

The most prevalent sperm defects detected during this study was Minor midpiece defects, loose heads, Proximal cytoplasmic droplets, head defects, and dag reflexes. The majority of these defects are of testicular origin and occur during the process of Spermatogenesis and may modify during epididymal transfer [1, 15, 43].



Graph 5: Different morphological abnormalities observed during semen evaluation

6. Summary

The population of the South White Rhinoceros specie is being challenged by various aspects of which poaching for their horn has the biggest influence on their numbers. Another factor is the bottleneck in gene diversity due to homogeneity between modern and historical samples of Natal rhinoceros. The conservation of these species may require intervention by breeding management and various assisted reproductive technologies (ART).

In this study, several ejaculates were collected from 7 Southern White Rhinoceros individuals. Four rhinoceroses were extensively free-ranging, and 3 had shelter and access to natural veld in the semi-intensive group. The ejaculate samples were collected by electroejaculation. In field conditions, basic semen analysis techniques were used to evaluate the semen including macroscopic and microscopic evaluations of individual sperm cells.
The average volume of ejaculate per rhinoceros during this study was 53.75 ± 47.95ml with an average of 3.68 ± 3.16ml ejaculate per stimulation. The ejaculate predominantly a milky consistency with a white colour. The mass motility of the 7 rhinoceroses was 3.5, with the progressive motility of the sperm being 69%. The sperm had a 70% normal morphology.
Of all the parameters evaluated, the extensive free-ranging group had better semen quality except for normal semen morphology which was similar in both groups. The better semen quality in the extensive group may be explained by the lack of hierarchical dynamics experienced.

The data collected in this paper and the insight it provides into the normal semen quality of free-ranging Southern White Rhinoceroses can be used in future investigations in assisted reproduction technologies to conserve the species for the future.

7. Acknowledgments

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Thesis progress report for veterinary students

Name of student: Herman Jakobus Smith

Neptun code of the student: C8VTWF

Name and title of the supervisor: Dr. Horváth András

Department: Department of Obstetrics and Farm Animal Medicine Clinic

Thesis title: Normal Semen Quality of Free-ranging Southern White Rhinoceros in South Africa

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	02	06	Discussion of the topic, Title, and the content requirements of the thesis	
2.	2024	02	27	Introduction, checking and discussing the writing of objectives	
3.	2024	04	03	The process of finding resources, using useful websites and books	
4.	2024	04	12	Discussing how to make the literature background and how to make the materials and methods	
5.	2024	06	06	Improving and discussing the literature review	

Grade achieved at the end of the first semester: 5

Consultation – 2nd semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	09	09	Brainstorm on how to use results collected	
2.	2024	09	19	Talk about the discussion	
3.	2024	10	03	Discussed how to phrase the abstract and summary	
4.	2024	10	28	Second draft discussion	
5.	2024	11	26	Final Draft discussion	

Grade achieved at the end of the second semester: 5

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....

signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in.....