

Impact of Age on Sperm Parameters in Infertile Patients at the Laboratory of Clinical Cytology, Cytogenetics and Reproductive Biology of HALD in Senegal: A Preliminary Study

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Abstract

Background: In Africa, procreation is not only an individual desire; it is sometimes a family and social constraint, as it is considered an essential element of socialization. In Senegal, male responsibility in couple infertility is twice as high as that of women. Numerous studies have shown that male age significantly influences semen parameters. However, no study has yet evaluated the impact of age on semen quality among men residing in Senegal. **Objective:** To establish the cytospermiological profile of infertile men at the Aristide Le Dantec University Hospital and to assess the effect of age on sperm parameters. **Methods:** This was a retrospective, descriptive study with an analytical aim, conducted between January 2020 and July 2022. It included patients who underwent semen analysis as part of an infertility work-up. Semen analysis was performed in accordance with the 2021 World Health Organization guidelines. Linear regression testing was used to assess the relationship between the different variables. **Results:** A total of 628 patients were included. The mean age was 37.5 years, with a range of 17 to 68 years. The most frequent semen profile was oligoasthenoteratonecrospermia (18.15%), followed by azoospermia (13.7%). A decline in sperm parameters was observed in men over 40 years of age. **Conclusion:** Male age was found to have a significant influence on semen quality.

Keywords

Senegal, Male Infertility, Semen Analysis, Age

1. Introduction

Infertility is a matter of growing concern for couples. Its prevalence varies little from one region to another, and rates are comparable whether countries are high-, middle- or low-income. It represents a genuine health problem in every country and every region of the world, as it does not discriminate. It is defined as the inability of a couple to spontaneously achieve pregnancy after twelve months of regular unprotected intercourse [1]. Global estimates show that between 48.5 and 72.4 million couples suffer from infertility [2] [3]. Approximately 12.6% to 17.5% of couples of reproductive age are affected by infertility, with a higher prevalence in certain regions such as the Americas, the Western Pacific, Africa and Europe [4]. Male factors contribute to about 50% of cases, acting as the sole contributor in 20% of infertility cases and contributing jointly with a female factor in about 30% of cases [5]-[7]. These male infertility factors are generally defined by the results of the semen analysis (spermogram), a test whose purpose is to accurately assess semen quality. Male infertility is mainly associated with a disruption of spermatogenesis, leading to both quantitative and qualitative abnormalities in the semen such as oligo- and/or astheno- and/or teratozoospermia, and more rarely azoospermia [8]. Studies conducted in various countries around the world—China, North America, Europe, etc.—have shown a trend toward declining semen quality, notably a decrease in sperm count and in the number of young men with morphologically normal spermatozoa [9]-[11].

In Africa, and in Senegal in particular, procreation is not only an individual desire; it is sometimes a familial and social obligation, as it is regarded as an essential element of socialization. Little data exists on infertility in Senegal. Nevertheless, the most recent statistical data available are those from the Continuous Demographic and Health Survey published in 2013 (EDS-Continue 2012-2013), which reveals a low prevalence of primary infertility in Senegal—namely 2.5% of women in a union and 4.8% of all women. However, this survey provides no figures on secondary infertility rates or on male infertility, whether primary or secondary [12]. Rare retrospective studies on the epidemiological profile of male infertility published in 2006 and 2009 showed primary male infertility of between 66.5% and 68.4%, with male responsibility twice as high as that of the female—namely 31.7%—with a polymorphic spermiological profile [13] [14]. In Senegal, a study of 4636 Senegalese couples between 1983 and 1996 showed that in at least 50% of cases infertility was due to the male partner [15].

In the United States, a study assessing the effects of aging on the percentage of infertile couples showed that from the late thirties onward the man's age became an important factor, and the failure rate of conception after 12 cycles rose from 18% to 28% between 35 and 40 years of age [16]. In Africa, a meta-analysis conducted between 1965 and 2015 covering 14 articles from 5 African countries (Nigeria, Libya, Tunisia, Egypt and Tanzania) showed a decline in sperm parameters (sperm concentration) of 72% of its mean over the last 50 years, independently of variations in the mean age of the studied cohorts [17]. The majority of individuals

included in these studies come from Western countries or, rarely, from neighbouring African countries. It is in this context that this work was undertaken, with a view to establishing the various cytospermiological profiles of the infertile man at the Aristide Le Dantec University Hospital and to assessing the effect of age on sperm parameters, in order to generate important data for planning and interventions in medically assisted reproduction.

2. Materials and Methods

2.1. Type of Study and Data Collection

This was a retrospective, descriptive study with an analytical aim. The data were extracted from the semen analysis reports of patients who presented to the Laboratory of Clinical Cytology, Cytogenetics, and Reproductive Biology at the Aristide Le Dantec University Hospital (HALD) in Dakar over a 30-month period (January 2020 to July 2022). All patients seen as part of an infertility work-up, who were in a couple, and whose records were complete were included. Patients referred for pre-chemotherapy assessment and those with incomplete records were excluded from the study. The data collection forms, on which the information was recorded, were kept in a secure location. Strict confidentiality and anonymity were maintained throughout the process. Written informed consent for research purposes was obtained from each participant.

2.2. Semen Analysis

Semen samples were obtained by masturbation on the day of analysis, after disinfection of the glans and hands, with an abstinence period of between 3 and 5 days, and were analyzed in accordance with the recommendations of the World Health Organization (WHO, 2021). The semen samples allowed assessment of semen volume, motility, vitality, concentration, sperm morphology and the frequency of sperm abnormalities. The results were interpreted using the reference values of the WHO 2021 guidelines [18]. These values are as follows:

- pH: 7.2 or more;
- Volume: 1.4 mL or more;
- Concentration: 16 million spermatozoa or more per mL;
- Progressive motility: 30% or more;
- Vitality: 54% or more live spermatozoa;
- Round cells: fewer than one million per mL;
- Typical forms: 4% or more (Kruger score).

2.3. Statistical Analysis

The extracted data were entered using Excel 2021 software and then analyzed using RStudio software. In descriptive analysis, variables were presented as percentage, frequency, mean and standard deviation. Separate simple linear regression models were used to examine the relationship between age and certain semen parameters (semen volume, sperm concentration, motility, vitality and sperm mor-

phology). Model fit was assessed using the coefficient of determination (R^2) and the overall F-test. In addition, the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) were calculated, and the best model was selected for each variable on the basis of these criteria. The difference was considered significant when $p \leq 0.05$.

3. Results

3.1. Characteristics of the Study Population

A total of 628 patient semen results were extracted during the study and distributed across 6 age groups. The mean age of patients was 37.5 ± 9.28 years, with extremes of 17 and 68 years. The predominant age group was [31 - 40] years at 43.31% ($n = 272$), followed by patients aged [41 - 50] years at 24.20% ($n = 152$) (Figure 1).

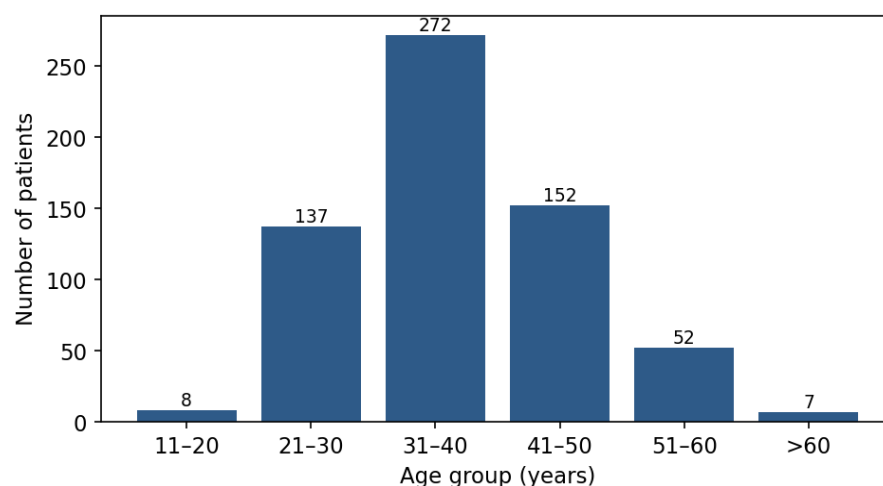


Figure 1. Distribution of the population by age group.

3.2. Semen Volume

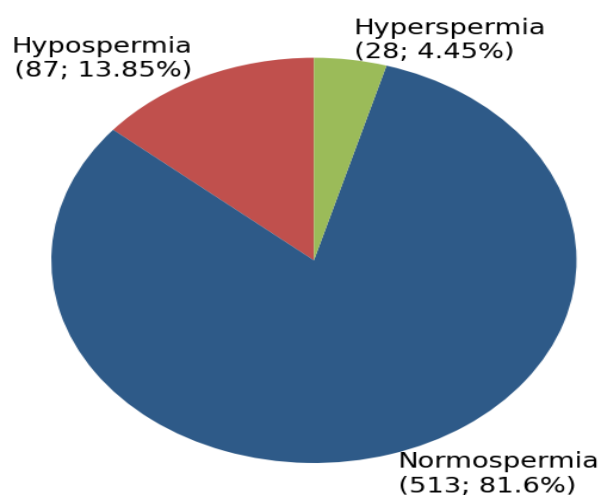


Figure 2. Distribution of patients according to semen volume.

The mean volume of the semen analyzed in our study was 2.76 ± 1.38 mL, with a minimum of 0.3 mL and a maximum of 8.23 mL. More than three-quarters of the patients—namely 81.6%—had normospermia (**Figure 2**).

3.3. Progressive Sperm Motility

Nearly half of the patients—namely 41.08% ($n = 258$)—had asthenozoospermia (progressive motility $< 30\%$), whether isolated or associated with another abnormality (**Figure 3**).

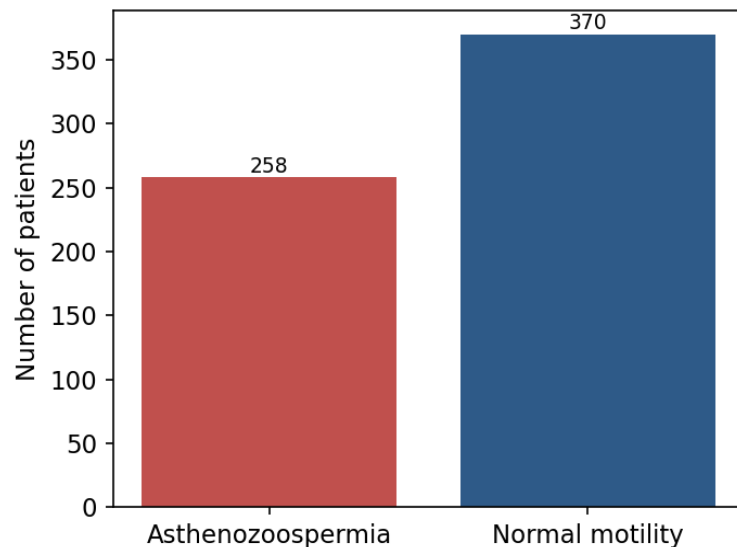


Figure 3. Distribution of progressively motile spermatozoa.

3.4. Sperm Count

3.4.1. Sperm Concentration

Analysis of the round cells showed leucospermia in 28.68% of cases ($n = 176$).

The mean sperm concentration of the semen analyzed was 39.57 ± 41.08 million/mL, with extremes ranging from 0 to 226 million spermatozoa/mL of semen.

Among the abnormalities observed, oligozoospermia was the most frequent at 26.11% of cases, followed by azoospermia at 13.69% of cases; cryptozoospermia accounted for 3.18% of patients (**Table 1**).

Table 1. Sperm concentration of patients.

Concentration	Count	Percentage (%)
Azoospermia	86	13.69
Cryptozoospermia	20	3.18
Oligozoospermia	164	26.11
Normozoospermia	358	57.01

3.4.2. Sperm Vitality

Analysis of vitality showed that nearly half of the patients—namely 46.18% ($n =$

290)—had necrozoospermia, and in five (5) of these patients this necrozoospermia was associated with leucospermia.

3.4.3. Sperm Morphology

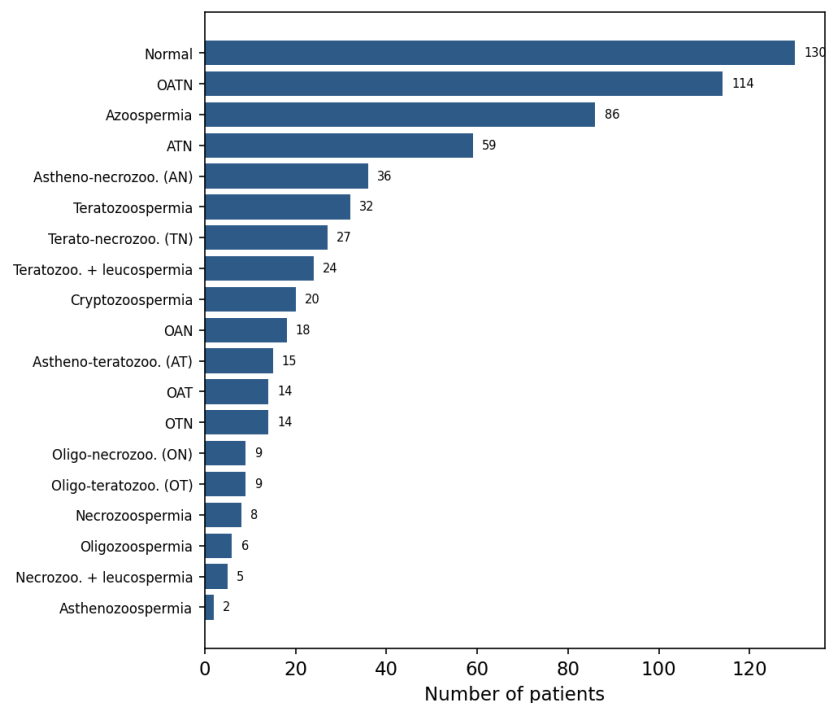
Analysis of sperm morphology on the spermocytogram showed that nearly half of the patients had teratozoospermia (typical forms < 4%) (**Table 2**).

Table 2. Sperm morphology of patients according to Kruger criteria.

Morphology	Count	Percentage (%)
Teratozoospermia	308	49.04
Normal morphology	320	50.96

3.5. Profile of Sperm Abnormalities

Analysis of the spermiological results shows that one-quarter of the study population had a normal result. Oligoasthenoteratonezoospermia was the most frequent abnormality at 18.15% of cases ($n = 114$), followed by azoospermia at 13.7% ($n = 86$). Isolated teratozoospermia, necrozoospermia and asthenozoospermia represented respectively 5.1%, 1.27% and 0.32% of patients. Associated abnormalities accounted for 54.78% of the abnormalities observed (**Figure 4**).



Note: OATN: oligoasthenoteratonecrozoospermia; TN: terato-necrozoospermia; OAN: oligoasthenonecrozoospermia; ATN: asthenoteratonecrozoospermia; AT: asthenoteratozoospermia; OAT: oligoasthenoteratozoospermia; AN: asthenonecrozoospermia; OTN: oligoteratonecrozoospermia; ON: oligonecrozoospermia; OT: oligoteratozoospermia; Necrozoo.: necrozoospermia.

Figure 4. Semen abnormality profile of the study population.

3.6. Trends of Age on the Quality of Sperm Parameters

3.6.1. Effect of Age on Semen Volume

The [31 - 40 years], [21 - 30 years] and [41 - 50 years] age groups had a mean semen volume of 2.94 mL, 2.81 mL and 2.49 mL, respectively. The over-60 age group had the lowest mean volume, at 2.10 mL. A significant association was found between increasing patient age and a decrease in semen volume, with a regression coefficient of -0.018 (95% CI: -0.029 to -0.006 ; $p = 0.007$) (**Figure 5**).

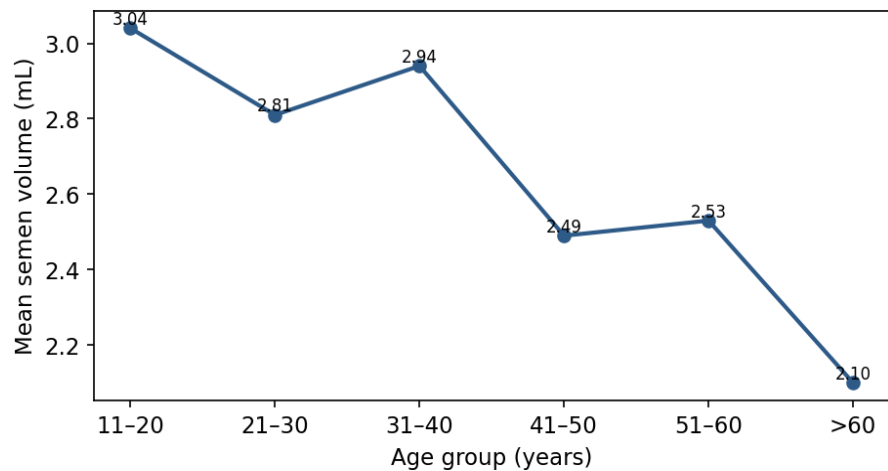


Figure 5. Mean ejaculate volume by age group.

3.6.2. Effect of Age on Sperm Concentration

The age groups with the highest sperm concentrations were [21 - 30 years] followed by [31 - 40 years], with mean concentrations of 46.97 million and 38.69 million spermatozoa/mL, respectively. Age had a significant impact on sperm concentration, which decreased by 0.338 million spermatozoa/mL per year of age (95% CI: -0.73 to -0.054 ; $p = 0.0001$) (**Figure 6**).

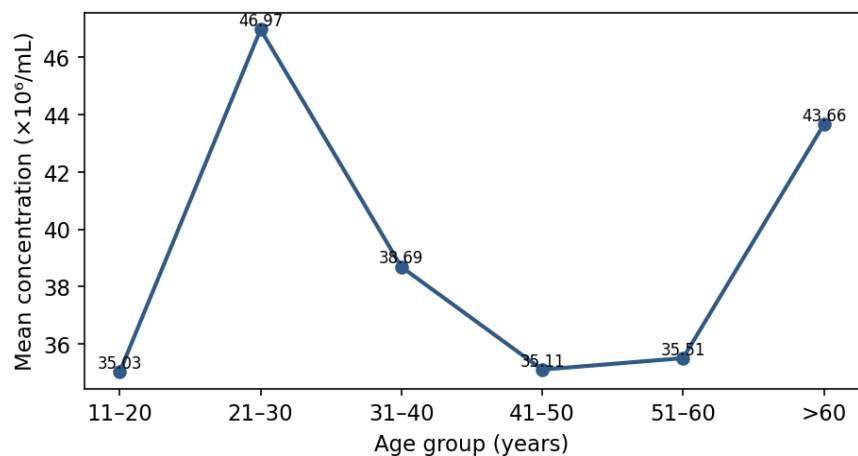


Figure 6. Mean sperm concentration by age group.

3.6.3. Effect of Age on Sperm Motility

The [21 - 30 years], [31 - 40 years] and [41 - 50 years] age groups had a mean mo-

tility well above 30%. Indeed, patients aged 31 - 40 years had a mean motility of 40%, compared with those aged 41 - 50 years at 38% mean motility after 30 minutes. Patients aged 21 - 30 years had a mean motility of 45%. A correlation between age and sperm motility was found, with a decrease of 0.2% (95% CI: -0.003 to 0.000; $p = 0.0034$) (**Figure 7**).

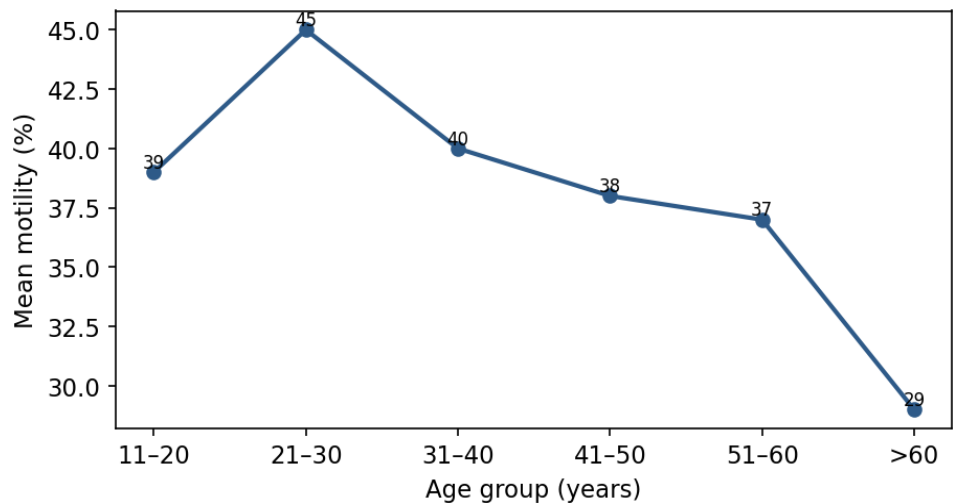


Figure 7. Mean motility by age group.

3.6.4. Effect of Age on Sperm Vitality

A sharp decline in vitality is observed in men over 40, although this decrease in vitality began in men over 30. A significant correlation of age on sperm vitality was found, with a decrease of 0.15% ($r = -0.15$; $p = 0.001$) (**Figure 8**).

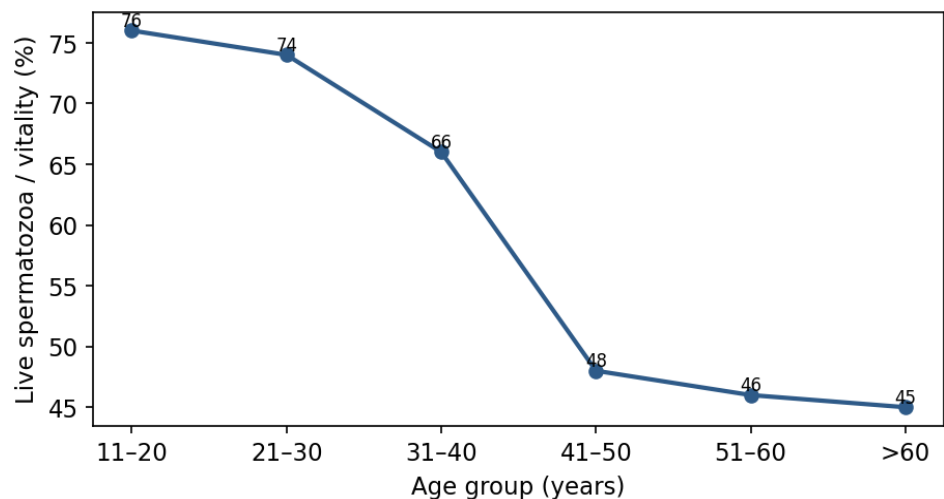


Figure 8. Mean live spermatozoa by age group.

3.6.5. Effect of Age on Morphology

From the age of 41 onward, a significant effect of age on typical forms was observed, with a coefficient of 0.19% (95% CI: -0.2067 to 0.02967), and a positive correlation was found between age and atypical forms ($p = 0.003$) (**Figure 9**).

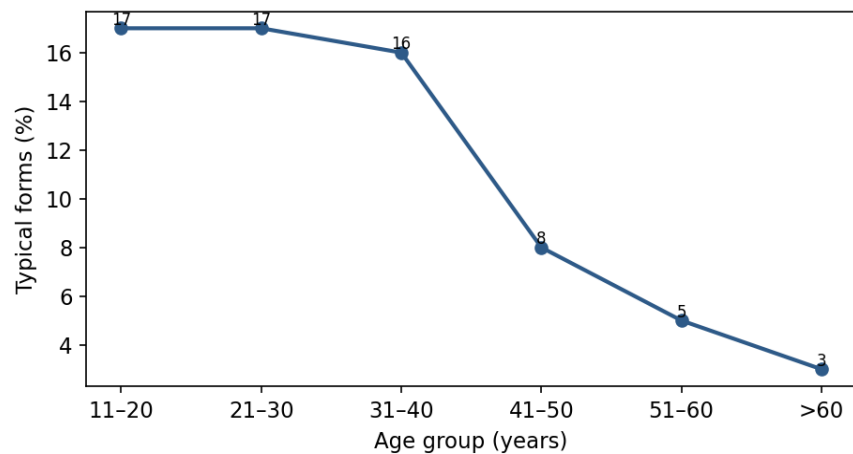


Figure 9. Mean typical forms by age group.

4. Discussion

4.1. Age of the Population

In a study population of 628 patients, the mean age was 37.5 ± 9.28 years, and the predominant age group was 31 - 40 years at 43.31% of cases, followed by 41 - 50 years at 24.2% of cases. Studies conducted in Senegal by Diao *et al.* (2006), Niang *et al.* (2009), Faye *et al.* (2000), Diallo *et al.* (2015), and Kane (2021) had already found that the mean age of patients consulting for a spermogram was between 30 and 45 years [13]-[15] [19] [20]. Similarly, elsewhere—in Morocco with Kbirou *et al.* in 2021, in Algeria with Bouchelaghem in 2017, in the DRC with Kadima *et al.* in 2023, and in Niger with Halidou *et al.* in 2022—the mean age of patients was 37.5 years, 37.4 years and 36.39 years respectively, with a predominance of patients aged 30 - 39 years (35.9%) and 40 - 49 years (24.54%), and 34 years with patients aged 30 - 40 years most frequently observed [21]-[24]. This mean age could be explained by the psychological complex some men feel about their infertility and therefore about consulting. But it also reflects that infertility would concern an increasingly young population, since the most represented age group was 31 - 40 years, and it highlights the impact of the media and advances in communication about male infertility in recent years, which have convinced young men to consult early, as well as the fact that the desire to procreate generally arises at this age.

4.2. Sperm Parameters

Our results highlight an alteration of sperm parameters to varying degrees, in order of frequency: morphological abnormalities (49.04%), followed by vitality (46.18%), sperm concentration (42.99%) and motility (41.08%).

4.2.1. Volume

Normospermia in our study was observed in 81.6% of patients, while hypospermia was found in 14% of patients. These data are similar to those found in 2009 and 2015 in Senegal by Niang *et al.* and Diallo *et al.*, with hypospermia of 11.9% and 10.5%, respectively [14] [19]. This contrasts with the results of Diao *et al.* in 2006

in Senegal, Traore *et al.* in 2008 in Mali, and Kadima *et al.* in 2023 in the DRC, where high rates of hypospermia were recorded in 28%, 44% and 34.1% of patients, respectively, although semen volume remained normal in the majority of cases [13] [23] [25]. These results could be explained, on the one hand, by the variance in the semen analysis methods used—in our study, we used the WHO 2021 references, whereas some authors such as Diallo *et al.* used those of 2010, and Diao *et al.* as well as Traore *et al.* used the WHO 1999 human semen analysis standards. On the other hand, by a long abstinence period or by anatomical and functional impairments of the seminal glands and ducts in cases of hypospermia, since semen volume essentially reflects the secretory capacity of the seminal glands; and, having been unable to assay the biochemical markers, we unfortunately cannot give with precision the causes of the hypospermia. Although a downward trend in sperm parameters is currently demonstrated, this trend was not observed in our study regarding semen volume, which remained unchanged, as in Nigeria between 2010 and 2019 among infertile men [26].

4.2.2. Motility

Sperm motility is known to be an essential factor in male fertility. Infertile patients had asthenozoospermia, whether isolated or in association, in 41.08% of cases. This result is in line with those of Kadima *et al.*, who found asthenozoospermia at 60%, and Diallo *et al.* in 2015, who found that 74.4% of patients had low motility. This contrasts with the studies conducted by Halidou *et al.* in 2022, which showed asthenozoospermia at 6.9%. This rate could be explained by the analyses performed by Kadima *et al.*, who found semen infections in 20.5% of patients and sexually transmitted infections in 32.7% of patients, with a predominance of *Staphylococcus aureus* (39.9%) and *Neisseria gonorrhoeae* (20.9%); Diallo *et al.* found leucocytospermia in 57.8% of samples; likewise Schuppe *et al.* in Germany showed in 2017 that asymptomatic inflammations of the genital tract affecting the epididymis, as well as chronic prostatitis, were responsible for the reduction in progressive sperm motility [19] [23] [24] [27]. Indeed, leucocytospermia in our study was present in 28.68% of cases.

4.2.3. Vitality

In this study, 46.18% of patients had necrozoospermia. These results are close to those of Diallo *et al.*, who found a prevalence of 76.2% of necrozoospermia in a cohort of 262 patients, among whom 42.5% had varicoceles, 37.2% had urogenital infections and 17.7% had testicular trauma [19]. In our study, necrozoospermia was often associated with leucospermia, so the responsibility of urogenital infections is preponderant.

4.2.4. Concentration

Analysis of the various semen parameters showed that, with regard to concentration, the mean was 39.57 million spermatozoa/mL of semen. In more than a quarter of patients, oligozoospermia was found (26.11%), and azoospermia was estimated in 13.69% of patients. Our rates are lower than those of Diao *et al.* (2006),

who found oligozoospermia at 46.5%—isolated in 10% of cases—and azoospermia in 23% of cases. Halidou *et al.* in Niger, for their part, found azoospermia in 50% of patients. These rates would be explained by the results obtained on semen culture, which were positive in 59.38% of patients in Niger and in 21.3% of patients in Senegal. Moreover, in the study conducted by Diallo *et al.* in 2015, azoospermia was estimated at 14.5% and urogenital infections accounted for 37.2% of cases. According to Schuppe *et al.* (2017), about 10% of men who have had acute epididymitis subsequently develop persistent azoospermia and 30% develop oligozoospermia. However, the disruption of spermatogenesis may be due to various factors, including exposure to vibration, emotional stress, and an increase in scrotal temperature during prolonged sitting [13] [19] [24] [27] [28]. Indeed, sperm concentration decreased by 40% for a 1 °C increase above the median (CI: 8% - 71%) [29], and two hours of driving would raise scrotal temperature by 2 °C [30]. Male infertility often has multiple causes, and infectious causes are among the most common; the infectious agents involved are most often bacterial or viral, which can lead to inflammation and increased oxidative stress, thereby altering semen analysis parameters and increasing sperm DNA fragmentation [31].

4.2.5. Morphology

Nearly half of the patients (49.04%) had teratozoospermia. Our rate is lower than that found in 2015 by Diallo *et al.*, which was 80.9%. This variation in results would be explained, on the one hand, by the method used to assess sperm morphology. Indeed, in 2015, Diallo *et al.* morphology was assessed according in David scale where the percentage of normal forms was 32% or more (WHO, 2010 guidelines), whereas in our study, the percentage of the normal forms was 4% or more Kruger criteria (WHO, 2021 guidelines). On the other hand, intra-individual, inter-individual and inter-laboratory variation exists in the assessment of sperm morphology [19] [32] [33].

4.2.6. Cytospermiological Profile

The cytospermiological profile of the abnormalities showed a predominance of oligo-astheno-terato-necrozoospermia (18.15%), followed by azoospermia (13.69%), astheno-terato-necrozoospermia (9.4%) and astheno-necrozoospermia (5.73%). This profile is similar to that found by Diallo *et al.* (2015). Furthermore, abnormalities associated with necrozoospermia and/or teratozoospermia were dominant and associated with leucocytospermia. This profile reflects the frequency of urogenital infections in our countries [19].

4.3. Influence of Age on Sperm Parameters

Several factors are responsible for sperm alterations. A study carried out in India by Kumar *et al.* (2017) found that age had a negative effect on semen volume, motility and sperm morphology, but had no effect on sperm concentration [34]. These results are close to those observed in our study, where a negative correlation is found between age and vitality, but also between age and sperm concentration.

Other authors, however, find a significant effect of age on ejaculate volume in South Africa, and a moderate negative correlation of age on morphology and progressive motility in the semen of Nigerian and South African patients [26].

Patients over 35 years of age show a late impairment of semen volume and morphology, and an early impairment of motility, concentration and vitality. Count, motility and morphology were also decreased in the study by Kumar *et al.* (2017) [34]. Although maternal aging contributes significantly to human infertility and men continue to produce spermatozoa even at an advanced age, paternal age may also contribute to human infertility [35]. Thus, advanced paternal age has been implicated in an increased frequency of miscarriages, autosomal dominant disorders, aneuploidies and other diseases [36] [37]. It has also been associated with an increase in the prevalence of genomic and epigenomic abnormalities, which would explain infertility in patients with no alteration of sperm parameters and the failure rate of assisted reproduction measures in these patients [38]–[40]. The early impairment of motility, concentration and vitality leads us to suppose that other associated factors, not explored in our study, may be involved in the deterioration of male fertility in Africa (genital infections, exposure to pesticides and heavy metals, tobacco and alcohol consumption, etc.), and to recognize the increase in the infertility rate in our regions.

4.4. Limitations

Our study encountered several limitations related to its retrospective nature, notably:

The absence of data on medical history, the type of infertility and the patients' lifestyle.

5. Conclusion

Infertility has become a major public health problem. There is an age-related decline in semen quality. The most affected age group is 31 - 40 years; oligo-asthenoterato-necrozoospermia and azoospermia remain the most frequent. The emergence of new risk factors—notably environmental, social and occupational—will lead to an increase in its prevalence. The spermogram currently remains the cornerstone of the assessment of male infertility. Complementary biological tests (biochemistry of the seminal fluid, sperm DNA fragmentation test, hormonal work-up, etc.) are essential and must imperatively be integrated into routine examinations. To this end, greater collaboration should be established between general practitioners, gynecologists, endocrinologists, urologists and biologists, for the greater comfort of patients and/or couples affected by the problem of infertility, but also with a view to developing research in this field.

Author Contributions

R.K.I. and M.S. contributed to the design and to writing of the study. A.D., C.D. and S.B.N. contributed to data extraction, to the analysis and interpretation of the data.

N.D., M.V.G. and A.S.D. contributed to editing the manuscript. All authors contributed to revising the manuscript critically for important intellectual content.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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