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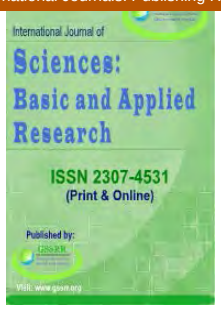

Risk Assessment of Male Infertility in People Aged 28 to 52, in the City of Parakou (Benin)

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Abstract

We evaluated male infertility in a moderately westernized population by macroscopic and microscopic examination. 29% of the population is free from difficulty conceiving; 34.90% has a relative difficulty to good mobility and 28.32% has a relative difficulty to the eligible number of sperm per ml. We found no correlation between the factors studied and the age of patients. Our work allows us to hypothesize the involvement of environmental causes among others knowing that in these environments the presence of heavy metals has been detected**.**

Keywords: Male infertility; Environmental causes.

1. Introduction

In African traditional societies, women have long been held responsible for reproductive difficulties of a couple. Male infertility has remained a taboo for religious or cultural reasons. But increasingly, the universalization of science and expansions of Western values has lifted the veil on the subject.

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Although many religious communities resist, it is increasingly common to see men get involved in the search for a medical solution due to reproductive difficulties. Data are increasingly available on male infertility, making that up to 50% of couples of reproductive difficulties are due to abnormalities [1].

Male infertility causes are many and varied. Human exposure to new chemicals such as heavy metals [2], temperature, exposure and ingestion of pesticides, new food consumption patterns are frequently found as causes, outside genital malformation.

In our study, we observed for several months that patients, who asked for feedback of their fertility, were becoming younger and male. Given the religious and traditional patterns of population of the city of Parakou, we found it useful to assess male infertility in patients manifesting problems conceiving for at least two years.

2. Materials and Methods

Materials: pH paper was provided by MERK, La Chapelle-sur-Erdre cedex – France. Formaldehyde solution was furnished by Roche Diagnostics, Meylan, France. The Marcano solution, Eosin Stain and the Lazarus solution were provided by LUCRON ELITECH Group, the Netherlands.

2.1 Sampling

Our panel is made of 80 patients who consulted a doctor for birth difficulty, during the 18 months of the study. A preliminary psychosocial study was conducted on the basis of a questionnaire filled by the patients.

2.2 Semen collection

The collection can be done in the laboratory or at home by masturbation or coitus interruptus, after at least 3 days of sexual abstinence and 7 days at most. The patient should not be treated with antibiotics. When the sample is taken out of the laboratory, the semen sample must be received no later than 60 minutes.

2.3 Macroscopic examinations

- Volume: This is the first parameter we appreciate before handling the sperm sample. All semen is slowly removed using a disposable syringe to assess the volume.
- PH: From a drop of sperm deposited on a pH paper, the value is read.
- Color: It will normally be a milky-white, white-ocher, cream or gray
- The smell: Chlorinated, foul (bleach smell).
- Viscosity: The viscosity of the liquefied semen can be assessed after gentle aspiration into a syringe adapted to a 21G needle, by selecting how the semen flows in the form of separate drops well. Sperm abnormal viscosity (increased) form filaments of more than 2 cm after each drop.

2.4 Microscopic examinations

- Sperm count: It is made using a MALASSEZ slide. We proceed initially to the dilution of sperm with a solution of formaldehyde or the MARCANO solution at 1:10, or more, depending on the sperm concentration. The dilution is proportional to the rate of spermatozoids.
- The leukocytes count: It is made using a MALASSEZ slide after a dilution of sperm with Lazarus solution at 1:20.
- The mobility: to assess the mobility, one drop of liquid and homogenized sperm is deposited using a syringe with a 21G needle on a door blade - own object and covered with a cover slip. The microscopic field is traversed and the mobility of sperm met each is classified according to the following categories: a) rapid and progressive mobility, b) slow or slightly progressive Mobility, c) non-progressive mobility (on site) and d) no mobility (immobility). 100 spermatozoa are counted in 4-6 fields and classified in the above categories with their respective percentages. The analysis is repeated. The final percentage of mobility is equal to the sum of the percentages of progressive mobility, i-e-s, a + b. Standard value ≥ 50% at the first hour of the broadcast and ≥ 40% in the second hour.
- The vitality: when the mobility rate is below 50%, the study of sperm vitality is required. The simple technique with eosin solution (0.5%, observed with 40x objective) and William test (0.5%, at 100x) are allowed for the study. The vitality is expressed as a percentage.
- Cytological study: this test assesses spermatozoa morphology, the percentage of typical shapes and that of atypical (abnormal forms) for the evaluation of male fertility.

2.5 The causes of errors

The results could be non- significant for one of these reasons: 1) incomplete Ejaculation; 2) Sexual Abstinence poorly or not complied with; 3) Sperm collection in condoms or unsuitable containers; 4) Mobility Study conducted not in time.

2.6 Statistics

Results are expressed as mean±SEM. The correlation between groups was evaluated with XLSTAT (Addinsoft, france). We first checked that the data for each group were normally distributed. We then carried out two-tailed Student's *t*-test or Pearson correlation.

3. Results

1) The age of the patients

The 80 patients who were chosen in this survey were aged 28 to 52 years. The average age is 32.36 ± 5.18 years, and the median age is 32 years.

2) Sperm sampling method

All patients gave their sperm by masturbation, with the exception of three who practiced a coitus interruptus.

3) The results of macroscopic examination

- The volume of the ejaculate: The average volume of semen collected in patients is 3.58 ± 1,85ml, with a median volume of 3ml. 80 taken on the patients, 17 provided a lower volume of sperm in 2 ml or 21.25%.
- The pH of semen: The average pH of semen samples is 7.35 ± 0.88. The median is 7.5. The collected data indicate that only two patients had a pH below the recommended range, and the other two have a pH above this range.
- The smell and color of semen: only one sample was not chlorinated characteristic odor, but a foul smell. Staining is almost always characteristic for a case but a yellowish brown sample was observed for another.
- The viscosity of semen: The results for the viscosity of semen samples are as follows: 76% normal, 13% fluid and 11% viscous.

4) The results of microscopic examinations

- Sperm motility: The results for the sperm mobility were measured during the first four hours. The results obtained are as follows:

Table1: Sperm mobility (in millimeters) during the first four hours after collection

	1st hour	2nd hour	3ird hour	4th hour
Mean (mm)	37.5696203	29.5822785	24.7594937	20.6582278
SD	25.9064805	21.8963804	20.3427144	18.9228434
Médian	35	30	20	15

These results show that the values are dispersed. Indeed, many samples have null sperm mobility. The results show that 61.25% of subjects have reduced sperm motility.

We researched the correlation between the first and the second hour motility of the sperm, figure 1 shows the correlation.

We also search for a relationship between sperm motility in the first hour and the patient's age. There is no direct relationship between these two parameters. Indeed, there is a null mobility both in subjects less 30 years old in subjects almost 40 years old. Similarly, the most motile sperm are those of subjects over 30 years old.

- The number of sperm per mm^3: Samples collected inform us that the subjects were on average 38,456 ± 4,700 sperm per semen mm3. The median value of the data collected is 25,500 sperm per mm^3. It is clear from these results that 32.5% of subjects have a lower sperm count per mm^3.

We observed the relationship between sperm count and age of the subjects. There is no direct link. In fact, a matter of twenty years possesses almost no sperm, while a patient older than 40 years old with more than 250,000 per mm^3.

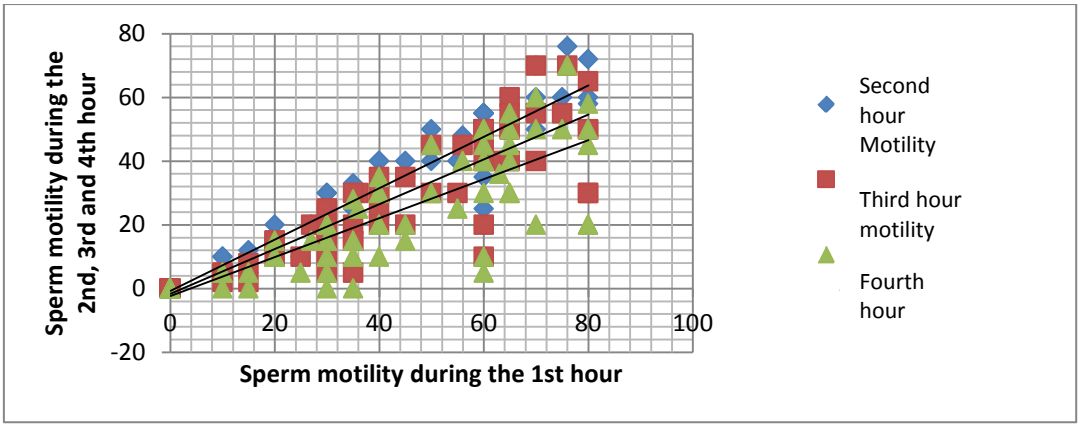

Figure 1: The correlation between the first hour sperm motility and the next four. The correlation is positive and direct. Sperm mobility beyond the first time depends on that observed during the first hour.

By relating the number of sperm and semen volume issued, we observe that there is no relationship between these two parameters.

The correlation between sperm count and mobility of the latter is not clearly defined (correlation coefficient r = 0.4 and P = 0.2).

- The number of white blood cells: The average number of white blood cells is 24219 ± 8279; the sample median value is 20000. Only 12.5% of the subjects have a normal white blood cell count above. There is no direct correlation with age of patients.

5) The immediate diagnosis

We have established an array of abnormalities diagnostic revealed by the analysis of semen samples of patients.

Table2: Risk of occurrence of sperm abnormalities'.

Modalities	Risk of occurrence
Normal	29%
Asthénozoospermia	16.25%
Oligozoospermia	17%
Asthénozoospermia and oligozoospermia	17.5%
Azoospermia	3.75%
Other pathologies	17.5%

We found only 29% of normal samples. The table resumes the abnormalities' due to motility, physical and chemical properties.

4. Discussion:

We have collected and analyzed semen samples from 80 individuals aged 28 to 52 years and residents in the city of Parakou. We performed macroscopic examination (color, odor, viscosity, pH ° and microscopic examinations (mobility, sperm count and white blood cells per milliliter).

The data inform us that there is no correlation between patient age and none of the parameters measured. Anomalies can occur at any age of the patient, and would rather environmental causes.

Procreation is seen as a cultural obligation in this region of Republic of Benin.

The part of man's responsibility in the etiology of infertility of a couple is established. This variable etiology dysfunction is justified in part by infections since in 13.93% of cases are accompanied by inflammation of sperm channels.

Sum all, only 29% of the population is free from difficulty conceiving. 34.90% has a relative difficulty to good mobility and 28.32% has a relative difficulty to the eligible number of sperm per ml.

Further work should to consider fertility status of our patient's wife to assess whether the causes of difficulties at birth are not from the partner or shared.

However, we point the finger at environmental causes to explain the anomalies observed in our male patients. Parakou is not a big city, the share of environmental pollution come from unsuspected substances such as heavy metals. Indeed, in West Africa, studies have reported the presence of heavy metals related to the gas market still lead in conditions devoid of any security measure [3] .The socio-psychological questionnaires inform us that 60% of our patients are supplied in this type of fuel outlets.

Alcohol, tobacco, overweight or obesity, are common causes of infertility. In addition, exposure to lead and cadmium are related to a fertility decline: male lead exposure is associated with a change in the characteristics sperm, increasing the risk of infertility involuntary. Cadmium, acting as a endocrine disruptor is also likely to influence fertility [4, 5]

Pesticides, persistent organic pollutants (dioxins, PCBs), solvents and ionizing radiation can also affect fertility perturbing spermatogenesis [6].

Some exposures may affect trans generational: the effects appear in children of exposed people or little time after exposure (case of defects) or several years later (cancer, infertility) [7].

Further work is underway to try to make a correlation between blood levels of heavy metals and the difficulties of birth on the one hand and a correlation between those difficulties and the body mass index on the other hand, among young people of the city of Parakou.

5. Conclusions

The couple's infertility is not a problem of only women. Through our work, we can learn that men have a good share of responsibility in a couple's infertility. This part of man's responsibility has various causes. The causes can be genetic, natural, infectious or environmental, such as exposure to endocrine disruptors.

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