

Short Communication

Andrology Laboratory Update

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- Semen analysis
- CASA
- Quality assurance programs

Abstract

Background: Increasing availability to assisted reproduction (AR) treatments and advances in cryobiology, mainly oocyte vitrification, modified andrology laboratories performance. On the other hand, environmental and lifestyle factors seem to have a deleterious effect on semen quality.

Aim: To evaluate the demand and evolution of seminal parameters in the last two decades, characterize the current andrology laboratory and assess its improvement after computer-assisted systems (CASA) inclusion and to appraise the adherence to quality assurance procedures.

Materials and methods: Data from 22,528 semen analysis from patients attending the Laboratory of Male Fertility at the Buenos Aires University Clinical Hospital "José de San Martín", were analyzed. Information regarding Semen Analysis External Quality Control Schemes (EQCS) in Argentina is reported.

Results: Overall demand decreased significantly while first-time ordered increased. Sperm volume and sperm count, androgen dependent parameters, went down over the years. Strict morphology criteria decreased the percentage of normal sperm. The CASA system allowed objectifying sperm kinetics, increasing the percentage of fast progressive sperm (Ga). The number of participant laboratories in Semen Analysis EQCS was 62 and 146 at present. Only four laboratories became accredited.

Clinical Translation: Male fertility could be at risk.

Strengths and Limitations: Two decades spermiograms results were recorded. The reported data are limited to Argentine

Conclusions: The Andrology Laboratory demand decreased due to an increase in AR treatments. Either sperm count or semen volume diminished over time. The decrease in the percentage of normal forms endangers its clinical value. Objective motility improves the assessment of kinetic parameters. Adherence to External Quality Programs improved although there are still very few accredited Andrology Laboratories.

INTRODUCTION

The first publication in reference to human fertility appeared more than fifty years ago [1], since then, the anthological laboratory became the cornerstone of the procedures offered. There were certain widely known landmarks that changed its course. With the advent of Assisted Fertilization techniques in 1978 and the recognition of its mentor Robert Edwards in 2010 with the Nobel Prize in Medicine [2], a new discipline "Clinical Embryology" [3] emerged. Currently there are more than 8 million children born by these techniques [4]. If we focus on male factor, gamete microinjection techniques inclusion turned out to be a revolutionary fact, solving previously inconceivable cases such as severe oligozoospermia or obstructive azoospermia [5].

The widespread diffusion of these procedures [6] impacted the demand for basic semen studies that include sperm motility, count and morphology. It should be noted that this is a functional test that requires an expertise observer to achieve the expected accuracy. A normal semen analysis results from adequate testicular function, mainly spermatogenesis, which is hormonally regulated. Thus, if the evaluated parameters show values within the expected range, it could be ruled out the endocrine factor. On the other hand, it could be asserted that there are no

obstructions, which would be evidenced by both the macroscopic and microscopic parameters [7]. The incorporation of Computer-Assisted Systems (CASA) made it possible to objectify the observations, keeping a record of all those [8].

The andrological laboratory is under the scope of the achieved advances in overall clinical biochemical laboratory. Adherence to quality assurance is growing, mainly External Quality Control Schemes (EQCS) and laboratory accreditation, resulting in precision reports improvement [9].

Environmental and lifestyle factors may have a deleterious effect on semen quality affecting laboratory results [10].

Our purpose is to analyze the demand and evolution of seminal parameters in the last two decades, characterize the current andrology laboratory and assess its improvement after computer-assisted systems (CASA) inclusion. Secondly we would like to appraise the adherence to quality assurance procedures.

MATERIALS AND METHODS

A total of 22,528 semen samples from patients attending the Laboratory of Male Fertility at the Buenos Aires University Clinical Hospital "José de San Martín", were analyzed. They emerged from the study of monthly means of internal quality

control taken from 2001 to 2019. Semen study was performed according to the current WHO Manual [11-14].

To assess sperm motility, the subjective method was employed until 2010; thereafter the CASA-SCA system (Sperm Class Analyser[®]-Microptic) [15] was used. Morphology was evaluated according to WHO 1992 criteria until 2006 when strict criteria (SC) replaced it in Papanicolaou stained smears. The immunological factor was evaluated using MAR-test (Mixed agglutination reaction test); at the beginning with an “in house technique” based on that reported by Jager [16], since 2019 the SpermMar Test-IgG (FertiPro) was used. DNA fragmentation was evaluated using the TUNEL technique (terminal deoxynucleotidyl transferase dUTP nick end labeling) [17].

The annual means of each parameter: volume (V), fast progressive motility (Ga), progressive motility (PR), total motility (TM) and morphology (N), were calculated as the mean of the monthly means and the trend line over time was determined; changes are expressed as: Pearson's coefficient (r); statistical significance ($p < 0.05$).

Argentine Biochemistry Foundation (FBA) Semen Analysis Subprogram for EQCS started in November 2005. The evaluated analytes are morphology, sperm count and motility. Two surveys each year are delivered to the participating laboratories, while immunological factor is evaluated once a year. The program follows the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

There are two independent bodies in refer to laboratory accreditation in Argentina according to the ISO Norm 15189 (Specifies requirements for quality and competence particular to medical laboratories) [18], the OAA (Organismo Argentino de Acreditación) and PAL (Programa de Acreditación de Laboratorios). We will report data on the last one in which we particularly worked.

RESULTS

Overall demand decreased between 2001 and 2019 significantly as shown in Figure 1 (-0.65; 0.003), while first-time ordered increased (0.83; <0.001) (Figure 2).

The results obtained from the trend lines for each parameter were: V (2001-2019) (-0.60; 0.007); Sp/ml (2001-2019) (-0.58; 0.01), Sp/ey (2001-2019) (-0.79; <0.001), Ga (2001-2019) (0.78; <0.001), Ga-subjective (2001- 2010)(0.36; NS), Ga-SCA (2010-2019) (0.28; NS), PR (2001-2019) (-0.59; 0.01), PR-SCA (2010-2019) (-0.53; NS), TM (2001-2019) (0.001, NS), TM-SCA (2010-2019) (-0.61, NS); N (2001-2019) (-0.83; <0.001), N-CE (2006-2019) (-0.71; 0.006) (Figures 3-5).

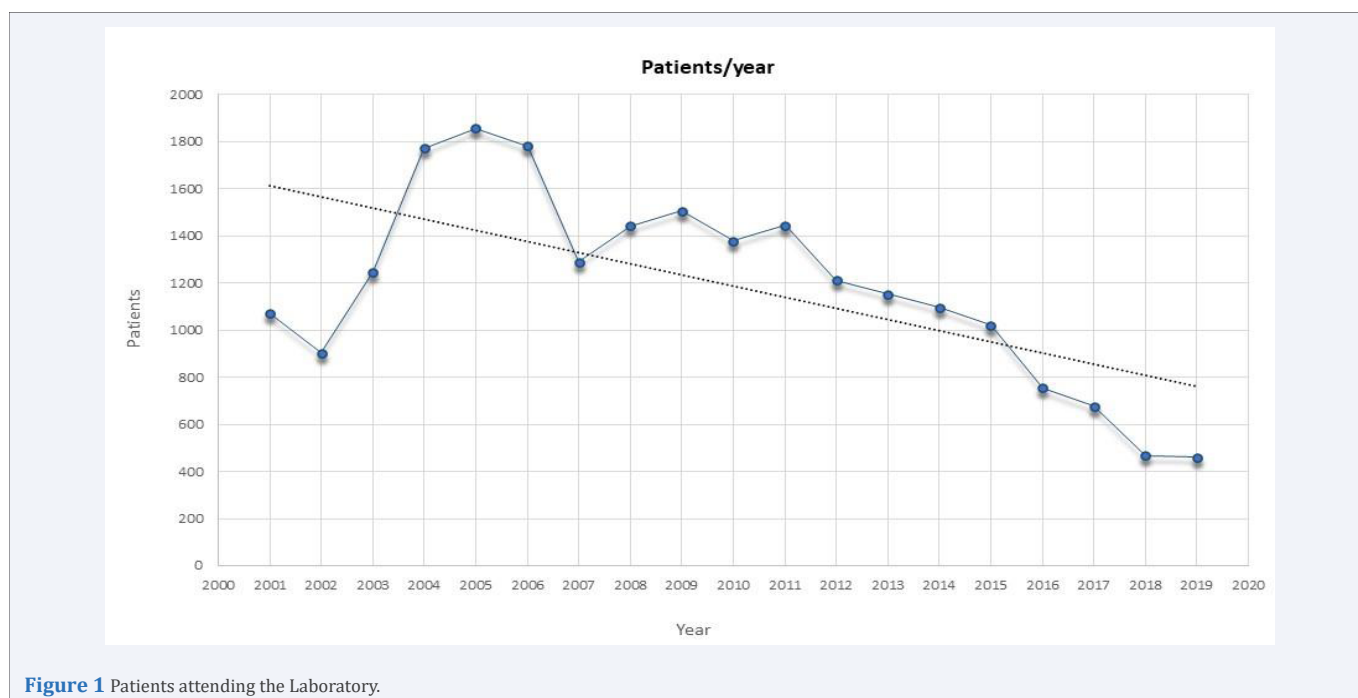
The MAR test and TUNEL requests were maintained over time: MAR test (0.28; NS); TUNEL (-0.18; NS).

The number of participant's laboratories increased steadily since 2005, when the Semen EQCS was created (Figure 6). At present 146 Laboratories are enrolled.

The Andrology Laboratory Accreditation Program started in 2017 in Argentina, only four laboratories achieved the expected standards since then.

DISCUSSION

Our laboratory, immersed in a teaching hospital, works since 1970 and was pioneer in semen study and andrology specialist training. In these last two decades the overall andrology laboratory demand diminished while first time ordered increased, probably due to greater accessibility to fertility treatments in Argentina after National Law of Assisted Fertilization [19] was promulgated and advances in cryobiology that expanded its scope to couples with female factor. The progressive and significant reduction in global demand with an increase in diagnosis defined by only one study denotes a paradigm shift in the treatment of male



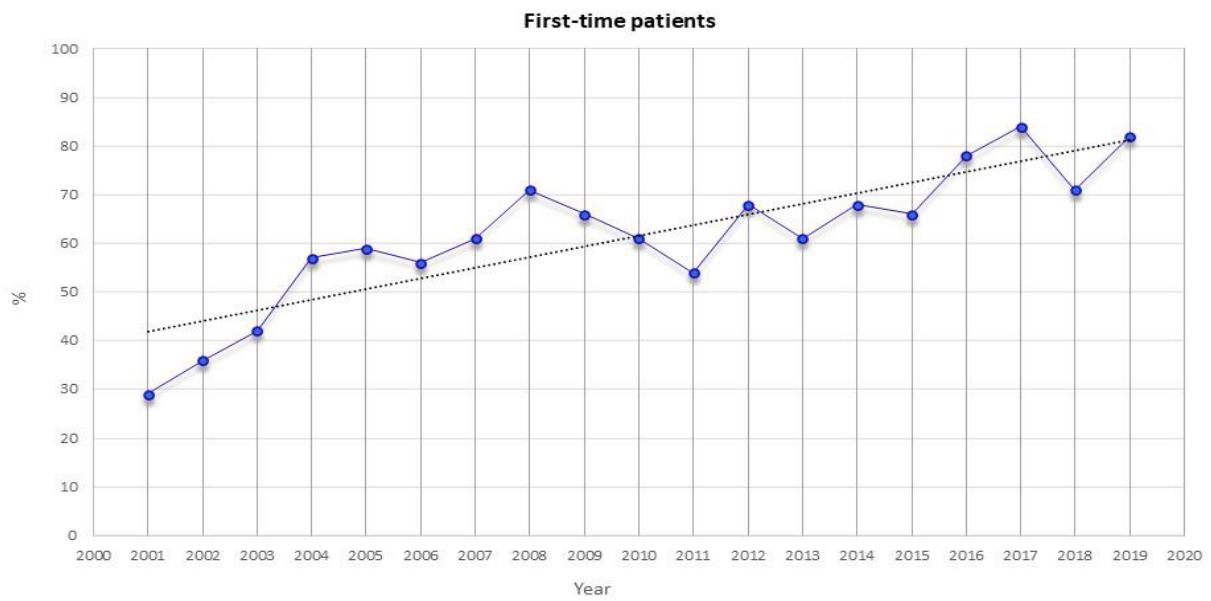


Figure 2 Patients diagnosed by only one study.

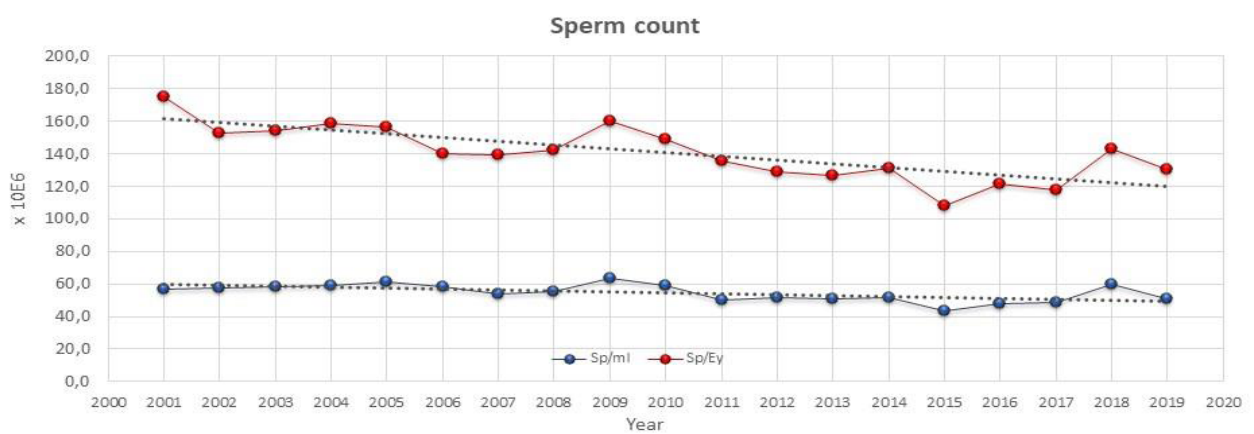


Figure 3 Evolution of sperm count over time. Sperm concentration (Sp/ml) and total number of spermatozoa per ejaculate (Sp/ey).

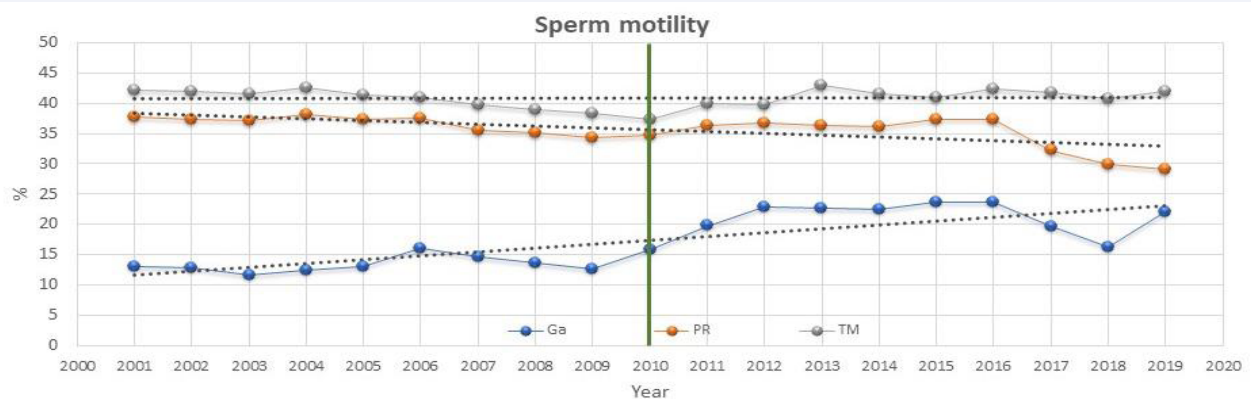


Figure 4 Evolution over time of sperm motility. Fast progressive motility (Ga), progressive motility (PR), total motility (TM). The vertical line indicates the change in methodology.

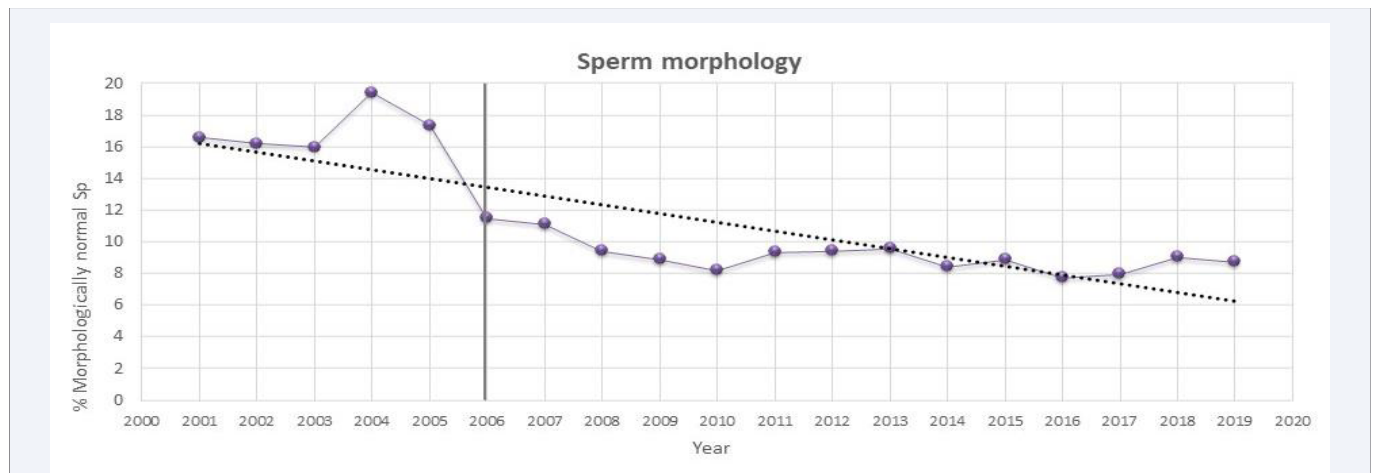


Figure 5 Evolution over time of sperm morphology. The vertical line indicates the change of criteria.

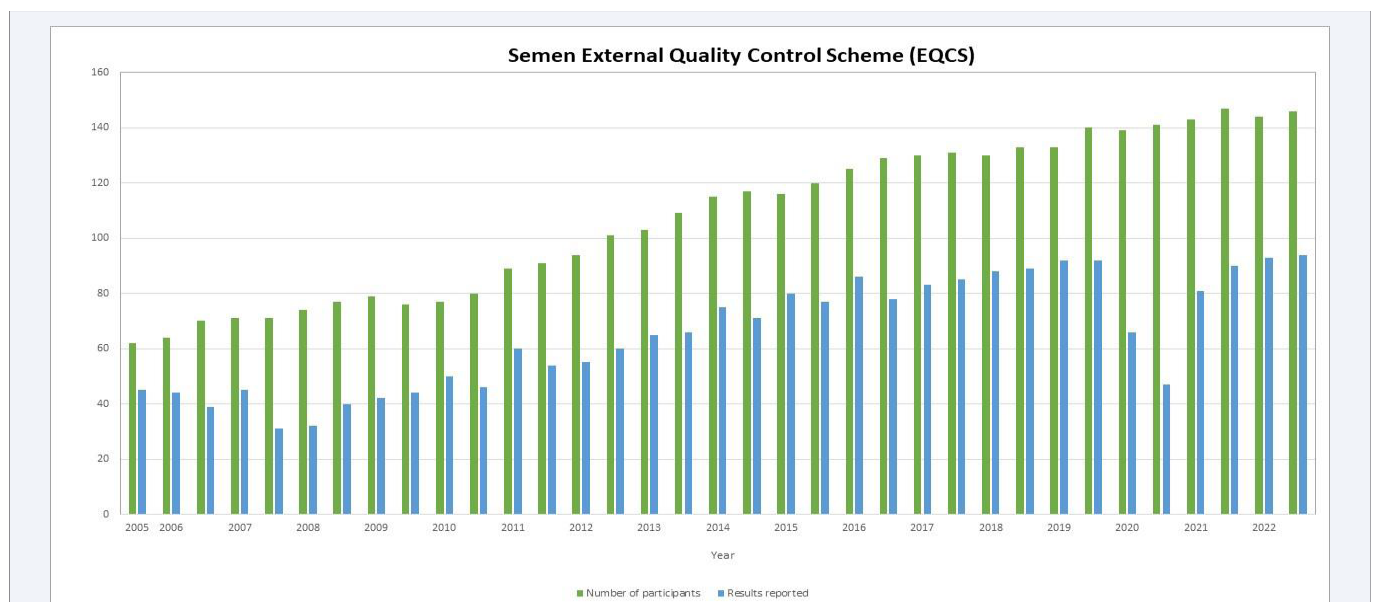


Figure 2 Evolution over time of the Semen Analysis EQCS.

factor, replacing medical and surgical procedures with assisted fertilization techniques.

The androgen-dependent parameters decreased over the years. We refer to sperm count and volume and attribute it to lifestyle changes that expose men to endocrine disruptors with epigenetic impact. A decrease in two million average sperm per ejaculate each year was observed in our population, data that are in accordance with those recently reported by Levine et al., [20].

There were two methodological milestones with an impact on the results. The first one was the incorporation of the strict criteria for morphology (2006), which decreased the percentage of normal forms [21]. The second was the acquisition of the CASA system (2010), which made it possible to objectify sperm speeds. The correct measurement of velocity allowed the sperm kinetics to be classified more accurately into its different motion characteristics (Ga, Gb, Gc and Gd subtypes), which was

evidenced by an increase in the percentage of rapidly progressive sperm (Ga).

We seriously believe that WHO Manual 2021[14] proposal to return to the old characterization of sperm according to their swimming velocity (Rapid Progressives $\geq 25\mu\text{m/s}$) is accurate. In our opinion, this parameter is essential, but it can only be evaluated using CASA [22] systems, since the subjective evaluation of these parameters, which requires estimation of swimming speed, is susceptible to errors and generates high coefficients of variation that ultimately end up in reducing the clinical value of the parameter [23].

Orders numbers for TUNEL test, for the study of DNA fragmentation, sustained over time, probably since it is considered a good predictor for "in vitro" fertilization outcome by the physicians.

Semen analysis is the first and main tool for evaluating male

fertility potential. The accuracy of the results depends on the employment of standardized methods, periodically audited and subjected to quality control. Uncontrolled techniques significantly influence the reliability of semen analysis results and may lead to delayed or inappropriate treatments.

CONCLUSION

In regard to Quality Assurance our results reflect a high Laboratory adherence to the Semen EQCS in spite of the fact that it is not mandatory in Argentina. Laboratories Accreditation [24] is a voluntary process in our country; it is very laborious and expensive. More beyond that it is yet not fully recognized by the health community. These statements may justify the low number of accredited laboratories shown in this report. In our opinion, it is a gradual educational process that tends to continuous improvement.

AUTHOR CONTRIBUTION STATEMENT

1. Bioq. Esp. Julia Irene Ariagno

Have made a substantial contribution to the design, data collection, analysis, and interpretation of data for the article.

2. Bioq. Esp. Patricia Chenlo

Have made a substantial contribution to the design, data collection, data analysis and drafting the article.

3. Bioq. Esp. Susana Curi

Have made a substantial contribution to the design, data analysis, drafting the article and critical revision of the article.

4. Bioq. Esp. Gabriela Mendeluk

Have made a substantial contribution to the design, data analysis, drafting the article and critical revision of the article. Approved the version to be published

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