SEMEN QUALITY AND AGING: ANALYSIS OF 9,168 SAMPLES IN CORDOBA. ARGENTINA

Rosa Isabel Molina¹, Ana Carolina Martini², Andrea Tissera¹, Jose Olmedo¹, Daniel Senestrari¹, Marta Fiol de Cuneo² and Ruben Daniel Ruiz².

¹Andrology and Reproduction Laboratory. Cordoba. Argentina.

Summary.- OBJECTIVES: Concomitantly with the actual trend towards later fathering, more detailed studies are necessary to establish the relationship between male age and seminal features.

The objective of the present paper was to evaluate the relationship of men age with semen quality and with the seminal levels of epididymal and accessory gland markers.

METHODS: The study was conducted as a retrospective study of 9168 cases obtained from the Andrology and Reproduction Laboratory in Cordoba, Argentina for 10 years (1995-2004) (men ages 20 to 77). An important number of factors such as abstinence time, toxic habits, work conditions and drugs consumption has been statistically considered. The parameters measured were: seminal volume, sperm concentration, total sperm count, sperm motility, morphology and viability. Seminal levels of alpha-glucosidase, fructose and citric acid were also evaluated.

RESULTS: We detected a significant decrease in seminal volume, sperm count, motility, viability and normal morphology, and a reduction in alpha-glucosidase and fructose levels in relation to age.

CONCLUSIONS: Since semen quality is a tool for fertility prognosis estimation, the weight of evidence indicates that men may become progressively less fertile as they get older. Couples who decide to delay childbearing should be warned about this matter.


Resumen.- OBJETIVO: En coincidencia con la actual tendencia hacia la paternidad tardía, son necesarios más estudios para establecer la relación entre la edad del varón y las características seminales.

El objetivo del presente trabajo fue evaluar la relación de la edad con la calidad espermática y con los niveles seminales de marcadores funcionales del epididímdo y de glándulas anexas.

MÉTODOS: este estudio retrospectivo fue realizado sobre 9168 casos obtenidos de hombres miembros de
Unlike women, men can generally conceive children beyond their 40’s. Men reproductive functions do not cease abruptly and spermatogenesis continue without any known critical threshold (1, 2). Moreover, despite a predictable decline in serum androgen levels in most individuals as age increases, serum total testosterone levels remain within the normal range in the majority of men (3). Nevertheless, some studies reveal that delayed fatherhood could impair the probability of conception, not only in couples consulting for infertility but in fertile couples too (4-6).

When exploring the bibliography related to this matter, the weight of the evidences suggests that increased male age is associated with a decrease in semen volume, in sperm motility and in the percentage of morphologically normal sperm, with no clear consensus about sperm concentration (1, 2, 7).

Although the mentioned parameters could be modified by altered function of the epididymis and/or accessory glands, only a small number of reports analyzed the seminal levels of markers such as alpha-glucosidase, fructose, zinc, and/or citric acid as evidences of deterioration in the function of these structures.

As well, while the influence of environmental toxic substances such as alcohol, tobacco, pesticides, drugs, etc on spermatogenesis is well recognized (8-13) only few studies take in consideration these habits as statistically controlled factors in order to avoid misreading of results.

Taken into account the trend towards later fathering, Kidd et al (2) claimed that more detailed quality studies are necessary to establish the relationship between male age and semen features; i.e., enrolling adequate numbers of men throughout the age spectrum, controlling for the effects of potential confounding factors and selecting appropriate comparison groups.

On the basis of the above stated, the purposes of the present study were to evaluate, in a large number of men attending an andrology laboratory in Córdoba (Argentina) for 10 years, the relationship of men age with semen quality and with the seminal levels of markers of epididymal, seminal vesicles and prostate function. To avoid misinterpretation of the results, an important number of factors such as abstinence time, toxic habits, work conditions and drugs consumption has been statistically also considered.

Semen samples were obtained from the male partner of couples being studied for infertility who attended the Andrology and Reproduction Laboratory (LAR) in Córdoba, Argentina. Data from 10 years (1995-2004) were analyzed. A form containing basic information and data about toxic habits, drug consumption and previous diseases was voluntarily filled out by patients. Incomplete forms, patients informing sexual abstinence outside the range (2-10 days), methods of collection different to masturbation and/or troubles during the collection procedure, were excluded from the study. Finally, in the present study 9168 semen samples (one per patient) were considered.

Seminal parameters evaluated

After liquefaction, semen analysis was performed according to the World Health Organization recommendations (14).
Seminal volume was determined in a graduated conic tube. Sperm concentration and motility were assessed by conventional methods in a Makler counting chamber (Sefi-Medical Instrument, Haifa, Israel) (15). Results were expressed as percentage of motile cells (A+B) (rapid plus slow spermatozoa). In the non-motile cell group, the viable spermatozoa population was evaluated with a supravital eosine technique (16) and the results were expressed as percentage of dead spermatozoa. The summatory of the percentages of rapid, slow, viable non-motile and dead non-motile spermatozoa comprise 100% of the gametes.

### TABLE I. GENERAL CHARACTERISTICS OF THE POPULATION ENROLLED IN THE STUDY; PATIENTS ATTENDING AN ANDROLOGY LABORATORY IN CORDOBA, ARGENTINE.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year of semen analysis</strong></td>
<td></td>
</tr>
<tr>
<td>1995-1996</td>
<td>12.6</td>
</tr>
<tr>
<td>1997-1998</td>
<td>19.6</td>
</tr>
<tr>
<td>1999-2000</td>
<td>21.1</td>
</tr>
<tr>
<td>2001-2002</td>
<td>20.3</td>
</tr>
<tr>
<td>2003-2004</td>
<td>26.5</td>
</tr>
<tr>
<td><strong>Abstinence (days)</strong></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14.4</td>
</tr>
<tr>
<td>3-5</td>
<td>68.8</td>
</tr>
<tr>
<td>6-10</td>
<td>16.8</td>
</tr>
<tr>
<td><strong>Urogenital pathologies</strong></td>
<td></td>
</tr>
<tr>
<td>Varicocele</td>
<td>22.0</td>
</tr>
<tr>
<td>Chriptorchidism</td>
<td>1.5</td>
</tr>
<tr>
<td>Inflammatory or infection disease</td>
<td>2.4</td>
</tr>
<tr>
<td>Urogenital surgery</td>
<td>11.5</td>
</tr>
<tr>
<td>Others</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Toxics exposure</strong></td>
<td></td>
</tr>
<tr>
<td>Tobacco</td>
<td>15.5</td>
</tr>
<tr>
<td>Alcohol</td>
<td>13.4</td>
</tr>
<tr>
<td>Others</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
</tr>
<tr>
<td>Nonsteroidal-antiinflammatory drugs</td>
<td>8.6</td>
</tr>
<tr>
<td>Hormones</td>
<td>0.9</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>0.3</td>
</tr>
<tr>
<td>Pentoxifilline</td>
<td>0.3</td>
</tr>
<tr>
<td>Others</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Sperm morphology was assessed employing the Papanicolaou staining and according to the WHO criteria (14).

**Statistical analysis**

The contrast of seminal parameter values between age groups was carried out with an Helmert inverse method in an analysis of variance (ANOVA), describing the results graphically for each age group as mean and their respective 95% confidence interval. In this test the mean of each age group is compared with the combined mean of all the previous groups (except the first). When necessary, natural scale of some sperm parameters were transformed to Gaussian distribution by square root or logarithm function.

The previous analysis was repeated including as covariable in the model each one of the possible factors of confusion considered (ANCOVA). In all cases, the bias resulted null or slightly significant. The confusion factors considered were: abstinence days, year in which the seminal analysis were performed, toxic and environmental exposition (tobacco, alcohol, pesticides, heavy metals, radiation and others), urogenital pathologies (such as varicocele, chryptorchidism, inflammatory or infection disease, urogenital surgery and others), and current medication (particularly nonsteroidal-antiinflammatory drugs, hormones, antibiotics, pentoxifilline, prescribed drugs for cardiovascular or diabetes diseases, psychotropics and others).

We apply an alpha value < 5% (p < 0.05) to consider reached the statistical significance level for reject the null hypothesis. In all cases, n represents the number of seminal samples evaluated. All the statistical analysis were performed with SPSS® 11.5.

**RESULTS**

Table I shows general characteristics of the population enrolled in the present study. The 9168 semen samples evaluated in the present study were grouped in age intervals as represented in Figure 1. The highest number of samples were obtained from patients aged 31-35 years old, whereby the most cases were included between 21 to 45 years old.

As can be seen in Figure 2, seminal quality diminished with age. Seminal volume decreased significantly in men aged more than 50 years and was parallel to a statistically significant decrease in total sperm count.

Sperm motility (rapid + slow motility) diminished whereas sperm viability (expressed as % of dead spermatozoa) decreased with age, both significantly starting with an age over 35. Since patients exhibiting sperm concentrations smaller than 0.15 millions were not considered, the number of samples evaluated in the above mentioned parameters was 8613.

The percentage of morphologically normal spermatozoa evaluated according to WHO criteria decreased significantly in men aged more than 50 years. Sperm morphology according to Kruger’s strict criteria displayed a similar profile (results not shown).

The results obtained from the biochemical evaluation of semen samples are presented in Figure 3. There were no differences in alpha-glucosidase levels in samples between 20 to 49 years old (62.45 mU/ejaculate; IC95% 60.9-64.0). From age 50 to 59, the seminal concentration of alpha-glucosidase reached a mean of 47.34 mU/ejaculate (IC95% 39.9-55.5) and diminished significantly with respect to the mean obtained from interval 20-49 years (p< 0.01).

Seminal fructose values also decreased with age; from age 45, results reached statistical significance, with a lineal diminution of 4.5 units/year (r= -0.131; p < 0.05).

Except for an unusual low mean value of citric acid on age ranging 50-54 years, no differences were obtained for this parameter in relation to age.
Seminal parameters evaluated according to different age ranges in patients attending an andrology laboratory in Cordoba, Argentine. Sperm morphology was quantified according to WHO recommendations. For motility and viability evaluation, semen samples with concentrations under $0.15 \times 10^6$/ml were not considered. Number of samples evaluated were: 9168 for semen volume and sperm count, 7167 for sperm morphology and 8613 for motility and viability. *: $p<0.001$ or 0.05 (for sperm motility) by inverse Helmert contrast.
No differences were detected in liquefaction time either (results not shown).

**DISCUSSION**

As Eskenazi et al (17) suggested, age-dependent alterations in semen parameters could be evoked by at least two broad ways of action. First, as it has been demonstrated for female fertility, there may be cellular or physiological changes in the genitourinary tract probably caused by age per se and/or by possible genetic mechanisms, i.e. morphological changes in the aging testis (6, 18, 19), decrease in the epididymal function, seminal vesicles and/or prostate (17, 20), changes in the levels of testosterone and related hormones (3) and decreased capacity to repair cellular or tissue damage. Second, age provides increased opportunities to suffer reproductive damages by factors such as urogenital infections, vascular diseases and/or the accumulation of toxic substances such as alcohol, tobacco, pesticides, etc (1, 4, 17).

Data from this study are robust because: a) the large number of men evaluated; b) the statistical correction of many confounding factors that was possible due to the large number of samples; c) the wide range of ages considered; d) the evaluation of seminal markers; such estimation provides valuable information about seminal quality and is not considered in most of the previous studies, e) the fact that Cordoba is a metropolitan area surrounded by country that join people with different life styles and habits and f) the fact that the results of the present study have been obtained from one laboratory and that all the measurements have been performed by only two well-trained technicians, in this way avoiding the methodological and experimental differences between laboratories (an inevitable feature of multicentric studies).

In accordance with most of the published reports (2, 7, 17, 21-27), in the present study we found that aging is characterized by a statistically significant decrease in semen volume, sperm count, motility and viability and an increase in the percentages of morphologically abnormal spermatozoa. Interestingly, semen quality in patients under 20 years tended to decrease (not statistically) with respect to age range 21 to 50. The reason for such a phenomenon should be further investigated.

With respect to seminal volume, the strongest known determinants of this parameter are the time since last ejaculation (28) and the seminal vesicle secretions, which provide the 70% of seminal plasma (20, 29). In the present study, the abstinence time was statistically controlled and this variable rises slightly.
but significantly with age. Considering the decrease in intercourse frequency with age, Ng et al (7) suggested that a reduced semen volume is more likely to be due to an impaired androgen action, accessory glands subclinical pathology and/or ejaculatory defects accumulated with age rather than to a reduction in abstinence intervals. In concordance with the above stated, when the seminal levels of fructose (a functional seminal vesicles marker) were evaluated, a significant and progressive decrease on ≥45 years old men was detected.

Considering that semen volume decrease with age, the fact that sperm density has not been found diminished by several authors is not surprising (7, 17), and does not prove that spermatogenesis remain unchanged. In this context, we should highlight the diminished total sperm count, which effectively has been demonstrated by us and other authors (7, 23). Because both, sperm morphology and gamete density reflects spermatogenesis (30, 31, 32), the simultaneous diminution in sperm density and the percentages of morphologically normal spermatozoa found in our study strongly suggests the occurrence of some level of impairment in the spermatogenic process.

With respect to sperm motility, we found a statistically significant decrease in this parameter starting on men aged 35, paralleled by an increase in the percentage of dead spermatozoa. The motility curve slope diminution is more evident in the last age interval (≥46) and was accompanied by lower seminal levels of neutral alpha-glucosidase, a marker of epididymal function, the structure where the spermatozoa acquire their motility (33). In concordance with our results, Henkel et al (34) found that in andrological patients and in sperm donors, age was negatively correlated with sperm motility and with plasma testosterone levels and positively with the percentage of abnormally stained flagella and flagellar zinc; both parameters reflect epididymal maturation. These authors postulated that since the epididymis is a functionally testosterone-dependent structure, sperm maturation could be altered in aging men. Nevertheless, Pasqualotto et al (23) informed a reduction in sperm motility concomitantly with age that was not accompanied by a modification in testosterone levels, suggesting that androgen deficiency is not the only cause of motility reduction. Since ours is a retrospective study, we could not measure this parameter.

It has been published that in older subjects the liquefaction time, a parameter depending on prostatic secretions (35), were prolonged when compared with younger subjects and suggested that this changes could impair the sperm motility. We neither found age-dependent alterations in liquefaction time nor differences in seminal citrate levels (except for an unusual low value at the age range 50-54).

**CONCLUSION**

Since semen quality is a tool for predicting fertility, the weight of evidences indicate that men may become progressively less fertile as they growth older. In their review, Kühnert & Nieschlag (1) concluded that although the effect of male aging is less prominent than the female one, men begins to contribute to the reduced fertility of a couple in their late thirties and to a reduced fecundity at the beginning of their forties. The tendencies detected in our study are in agreement with this observation. Couples who decide to delay childbearing should be warned about this matter.

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**REFERENCES AND RECOMMENDED READINGS**

(*of special interest, **of outstanding interest)


