



# The Uruguayan semen donor population: A twenty-eight-year retrospective study

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## Abstract

Several studies have reported a global decline in seminal quality over the years. The objective of this study was to describe the semen donor population of Uruguay through comparing data of successive samples banked by the same donors and the analysis of their semen and physical characteristics, ancestry origin and educational level. A total of 3,449 ejaculated samples collected from 71 donors, cryobanked between 1989 and March 2017 at Fertilab, were analysed. Results revealed a mean age of  $23.90 \pm 3.98$  years, an average weight of  $74.95 \pm 1.09$  kg and a mean height of  $1.78 \pm 0.06$  m. The majority of the donors trace their origin to Europe (74.65%, 53/71) and 66.19% (47/71) have a level of education higher than secondary school. We observed longitudinal differences in two parameters, that is sperm concentration and semen volume. Sperm concentration declined, while semen volume increased significantly over the 28-year period. The results of the present study are in accordance with that of previous articles that also reported a decline in sperm concentration over time. However, no differences were observed in total sperm number per ejaculate due to the increase in semen volume values, thus reflecting no real changes in sperm production over time.

## KEYWORDS

banking, cryopreservation, donor, spermatozoa

## 1 | INTRODUCTION

One of the critical steps performed in the andrology laboratory to help clinicians define the severity of male factor infertility is semen analysis (Nallella, Sharma, Aziz, & Agarwal, 2006). Variables evaluated during examination of semen samples include macroscopic (volume, viscosity, liquefaction and pH) and microscopic (concentration, motility, morphology and vitality) parameters. The protocols and recommendations provided by the World Health Organization (WHO) laboratory manual for the examination and processing of human semen are regarded as the gold standard for routine human semen examination, and these guidelines are globally used in the clinical

and research setting. The manual furthermore aims to provide reference values for the classification of men as fertile or sub-fertile. It was first published in 1980 and has been updated four times over a period of 30 years (Van der Horst & Du Plessis, 2017; World Health Organization, 2010). These updates were necessitated not only by a need to standardise and improve methodology and technology to evaluate semen quality, but also by the need for adjusting the reference limits according to the changes observed in seminal parameters of fertile men during the thirty-year period (1980–2010). Sperm concentration is one of the parameters considered of greater clinical relevance for the study of infertility. The first reference value was established in 1934 by Sandler (Sandler, 1953) who defined

the lower reference limit as  $60 \times 10^6/\text{ml}$ . In 1951, a new reference value was defined based on a study consisting of 1,000 males, and the updated value was significantly reduced to  $20 \times 10^6/\text{ml}$  since more than 95% of the fertile men who participated in that study had higher values (Macleod & Gold, 1951). The WHO only adopted this value in 1987. This value was maintained up to 2010, at which time evidence-based reference ranges and reference limits for various semen characteristics were adopted. The fifth version of the manual reported that the lower reference limit for concentration was to be lowered to  $15 \times 10^6/\text{ml}$ . This value was based on a study considering 4,500 men with proven fertility from 14 different countries and it was set as the 5th percentile (Cooper et al., 2009). The development and application of standardised protocols across laboratories and the establishment of lower reference limits largely assist in the successful diagnosis of infertile males. Therefore, these guidelines help to not only screen semen samples from men for both research and clinical purposes, but also allow and help to detect possible variations in semen parameters over time in the general population.

During recent years, several studies have reported a global decline in seminal quality (Jorgensen, 2001; Levine et al., 2017; Merzenich et al., 2010; Sengupta, Borges, Dutta, & Krajewska-Kulak, 2017; Willem, Ox, & Anssen, 2009). The observed fluctuations seem to be nonhomogeneous and huge variation in the semen quality of males from different regions have been reported (Auger & Jouannet, 1997; Jensen et al., 2001; Jorgensen, 2001), thereby supporting the idea that some environmental factors present in certain locations might be responsible for the changes observed in semen quality (Mendiola, Torres-cantero, & Agarwal, 2009). Lifestyle, environmental pollution and occupational exposure have been studied as possible causes of these observations (Bonde, Giwercman, & Ernst, 1996; Homan, Davies, & Norman, 2007). However, the results are contradictory and many critics negate this hypothesis as some studies have shown no decrease in semen quality in recent years (Benshushan, Shoshani, Paltiel, Schenker, & Lewin, 1997; Marimuthu, Kapilashrami, Misro, & Singh, 2003). Despite this, it is tempting to suggest that semen quality (specifically sperm count) is on the decline, and it is fair to say that male reproductive health appears to be under threat. Very little information is available on longitudinal studies on the semen quality in Latin American countries. To date, only a single study has evaluated sperm concentration, motility and morphology over a prolonged

period in a group of South American (Latin American) men. In this study, conducted between 1992 and 2003 in the city of São Paulo on 182 Brazilian semen donors, a significant reduction in sperm concentration and normal morphology was found (Glina et al., 2010).

Sperm donation was officially established for the first time in Uruguay in 1989 when Fertilab was founded. Until now, it is still the only heterologous sperm bank in Uruguay. The data collected during these nearly three decades represent a huge source of information regarding possible changes in sperm quality of the fertile Latin American and specifically the Uruguayan male population. Therefore, the objective of this research project was to study the entire semen donor population of Uruguay over the past 28 years by comparing data of successive semen samples banked by the same donors, as well as data regarding their physical characteristics, ancestry and educational level.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

This study received approval from the institutional ethics committee. A total of 3,449 ejaculated samples, collected from 71 donors and cryobanked between 1989 and March 2017 at Fertilab, were included in this study. In order to qualify as a donor, men had to comply with all the steps of the donor acceptance program (DAP). The Fertilab DAP consisted of three evaluation rounds, starting with an initial physical examination conducted by a physician and an interview during which the potential donor's family history, lifestyle habits and motivation for sperm donation are scrutinised. Once approved, this is followed by a semen analysis, cryotolerance test and swim-up test. Finally, blood, urine and semen samples are tested for infectious diseases, while a genetic screening is also performed.

### 2.2 | Semen sample analysis

Semen samples were analysed manually according to the WHO guidelines, based on the procedures described in the latest laboratory manual available at the time of sample examination (WHO

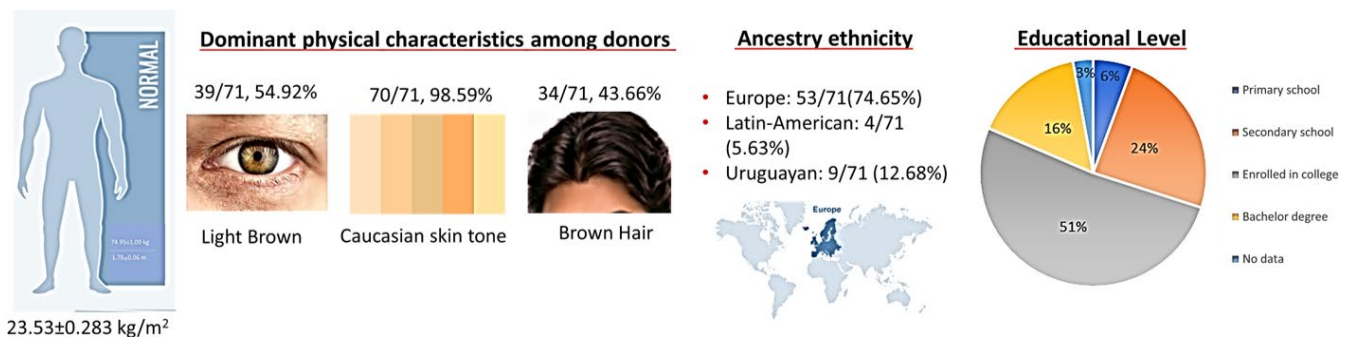


FIGURE 1 Physical characteristics and background information

1987, 1992, 1999 and 2010 guidelines). Concentration, volume, total sperm motility, progressive motility and vitality (by eosin–nigrosin) were recorded before cryopreservation.

### 2.3 | Semen cryopreservation

Ejaculates were cryopreserved according to the standard protocol followed at Fertilab (Ordoqui et al., 2018). Briefly, semen samples were divided into two equal parts and washed in Earle's balance salt solution (EBSS) without HEPES by centrifugation ( $300 \times g$ , 5 min). The supernatant was removed, and EBSS was added to the pellets in a single tube (final volume 0.5 ml). The freezing medium (TYB, Irvine Scientific) was added in a final ratio of 1:1. The samples were subsequently incubated for 20 min at room temperature (RT) before loading them into 0.25 ml straws. Subsequently, the straws were placed in liquid nitrogen ( $LN_2$ ) vapour for 20 min before finally submerging them in  $LN_2$  to be stored at  $-196^\circ C$ . After 1 min, a straw from each sample was thawed for cryotolerance testing. The frozen straws were placed in a pre-warmed conical tube at  $40^\circ C$  for 3 min. Thereafter, the content of the straw was expelled into a conical tube for immediate routine sperm analysis. Total sperm motility, progressive motility, nonprogressive motility and vitality were assessed post-thaw. Recovery rate (RR) for motility (i.e. proportion of initial motility retained after freeze-thaw) and recovery rate for viability (i.e. proportion of initial viability retained after freeze-thaw) were subsequently determined. A cryotolerance test was only performed on the first sample provided by each donor.

### 2.4 | Physical characteristics and background information

Information regarding educational level, paternity status, age and ethnicity, as well physical traits such as eye, hair and skin colour were extracted from the data collected during the donor's first interview. Each donor's height and weight were also used to calculate their body mass index (BMI).

### 2.5 | Statistical analysis

Statistical analysis was performed using XLSTAT software (Addinsoft, USA). Qualitative data are indicated in the form of relative frequency. The criteria for data normality were evaluated with the Shapiro–Wilk test. Comparison between groups was performed with a nonparametric Kruskal–Wallis test, followed by the Dunn's multiple comparison test. Data were expressed as median values and interquartile ranges, while means and standard error (SE) were also reported. In order to explain the relation between semen parameters and year of donation, the total donor population was clustered by number of samples banked/donor ( $<50$ ,  $50$ – $100$ ,  $>100$ ) as to evaluate whether semen features were affected by the number

of donations over the years. Spearman correlation coefficients were calculated between seminal parameters. Values of  $p$  less ( $<$ ) than .05 were considered statistically significant.

## 3 | RESULTS

### 3.1 | Physical characteristics and background information

As mentioned previously, the donor population comprised 71 men. At the time of initial screening, they had a mean age of  $23.90 \pm 3.98$  years. The average weight was  $74.95 \pm 1.09$  kg, and they had a mean height of  $1.78 \pm 0.06$  m. The calculated mean BMI value was  $23.53 \pm 0.283$   $kg/m^2$ , thereby indicating that the donor population was of normal weight with a P75 value equal to  $24.72$   $kg/m^2$ . The dominant physical characteristics among donors were light brown eyes (39/71, 54.92%), brown hair (34/71, 43.66%) and a Caucasian skin tone (70/71, 98.59%); only one donor had a dark skin tone. The ethnical origin of the vast majority of the donors is European (53/71, 74.65%), while 4/71 (5.63%) donors had Latin American ancestors and 9/71 (12.68%) identified themselves with Uruguayan ancestry only. Only 4/71 (5.63%) of donors reported to have merely finished primary school, while 17/71 (24.94%) completed secondary school. A further 36/71 (50.70%) were enrolled in college for an undergraduate programme, and 11/71 (15.49%) had a bachelor's degree. Two donors (2.81%) failed to answer this section. The majority did not have children at the time of enrolment (60/70, 85.71%), with 9 (12.85%) confirming biological paternity, while the data pertaining to this question were unrecorded for two donors (Figure 1).

### 3.2 | Donors' first semen sample and cryotolerance test

During the 28-year period (from 1989 to March 2017), a total of 71 males voluntarily donated semen samples at Fertilab. Table 1 summarises the descriptive statistics of the initial semen evaluation, including volume, concentration, total sperm count, normal sperm morphology and progressive motility for each donor. The cryotolerance test RR for viability and progressive motility are also listed in Table 1.

### 3.3 | Sperm parameter values over the 28 years

A total of 3,449 sperm samples were banked from 1989 to March 2017. Table 2 summarises the descriptive statistics of the sperm parameters from all the samples banked during the study period. On average, each donor banked  $48.57 \pm 3.99$  samples, and as expected, the mean values for each parameter exceeded the lower reference limits set by the latest WHO guidelines (Cooper et al., 2009). To indicate individual donors' contribution to the study, the number

**TABLE 1** Sperm parameters and recovery rates of donor's first sample from 1989 to 2017

Descriptive statistics	Volume (ml)	Concentration ( $\times 10^6$ /ml)	Total spermatozoa count ( $\times 10^6$ )	Progressive motility (%)	Normal morphology (%)	Viability RR (%)	Progressive motility RR (%)
<i>n</i>	71	71	71	71	63	60	64
Median	3.70	90.00	275.00	65.00	17.00	74.00	54.00
<i>P</i> <sub>25</sub>	2.50	61.98	203.96	58.00	14.00	61.50	44.75
<i>P</i> <sub>75</sub>	4.55	118.50	412.21	71.00	42.00	84.25	60.25

Abbreviation: RR, recovery rate.

of donations per donor is shown in Figure 2. Most of the donors (13/71, 18.31%) donated between 31 and 40 samples each. This was followed by 11/71 donors who performed 41–50 donations each. Only 6/71 men donated less than 10 samples, while 6/71 men were responsible for donating more than 100 samples each.

In the very first year (1989), only two men participated in the programme, providing six samples in total. Since then, an average of  $118.931 \pm 18.04$  donations were made/collected annually. The maximum number of donations per year ( $n = 411$ ) was received in 1993 from a total of 14 donors (Figure 3), while no donations occurred in 1999. The majority of our donor population banked samples within a two-year period, none of them banked during the entire studied period.

The samples were clustered in 7-year increments according to the year of collection. The medians, *P*<sub>25</sub>, *P*<sub>75</sub>, means and SE for the various parameters of these subgroups are displayed in Table 3 in order to try and establish a representative overview of possible changes/trends over time. Interestingly, the main changes observed over time were in sperm concentration and semen volume. Sperm concentration decreased significantly, while semen volume increased significantly over the 28-year period. A trend of an increase in progressive motility was also observed over time.

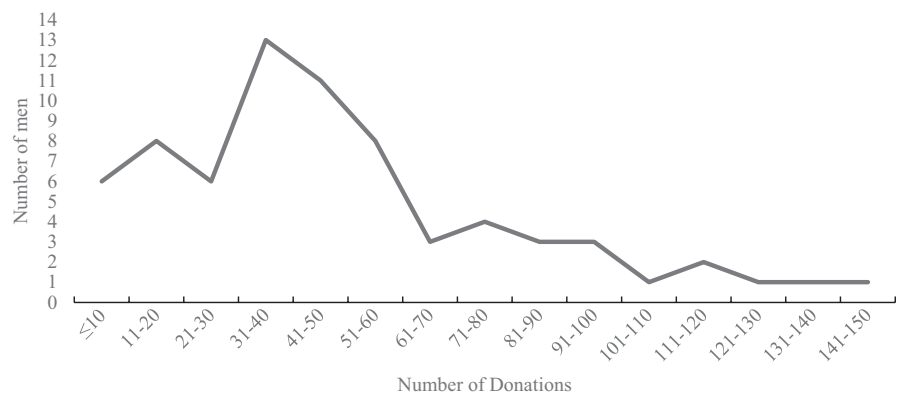
The samples were clustered into three groups according to number of samples banked per donor during their entire period of banking at Fertilib. Subgroups were divided as follows: donors who banked less or equal to 50 samples ( $\leq 50$ ); donors who banked between 50 and 100 samples (50–100); and donors who banked more than or equal to 100 samples ( $\geq 100$ ). The means and SE for the different semen parameters of these subgroups are displayed in Table 4 in order to try and establish a representative overview of possible changes in semen parameters caused by the number of donations. Donors who donated between 50 and 100 samples showed better results in sperm concentration, total motility and progressive motility. However, their semen volume was the lowest among the three subgroups. The reduction in ejaculate volume affected the number of total motile spermatozoa in this group, thereby showing the worst results among subgroups. Analysis of correlation was performed to quantify the linear association between sperm parameters and year of donation, and the results are shown in Table 5. A significant negative correlation was observed between concentration and volume ( $-0.242$ ,  $p < .001$ ). A negative correlation was also observed between concentration and year of donation for the groups  $\leq 50$ , and  $\geq 100$  samples, as well as for the totality of groups ( $\leq 50$ , 50–100 and  $\geq 100$ ). However, this trend was not significant for donors who banked between 50 and 100 samples. In order to describe the relation between variables, we plotted the results per donor/group in Figure 4. Despite a reduction in sperm concentration over the years, the variable of total sperm count was slightly increased ( $0.101$ ,  $p < .001$ ).

## 4 | DISCUSSION

The objective of this study was to describe the semen donor population of Uruguay through comparing data of successive samples banked by the same donors and the analysis of their semen and

**TABLE 2** Sperm parameter values of 3,449 donor samples from 1989 to 2017

Descriptive statistics	P <sub>25</sub>	P <sub>75</sub>	Mean	Median	SE
Samples per donor	28.00	65.50	48.57	41.00	3.99
Concentration ( $\times 10^6$ /ml)	60.00	110.00	85.70	85.00	0.63
Volume (ml)	2.00	3.80	3.03	2.60	0.02
Total sperm count ( $\times 10^6$ )	150	315	245.65	220	2.30
Progressive motility type A (A, %)	29.00	49.00	39.01	40.00	0.25
Progressive motility type (B, %)	18.00	34.00	25.79	22.00	0.19
Progressive motility (PR, %)	59.00	71.00	64.69	65.00	3.18
Total motility (PR + NP, %)	67.00	78.00	71.81	73.00	0.18
Total motile spermatozoa ( $\times 10^6$ )	109.80	232.87	179.56	160.60	173.93
Straws frozen/ejaculated	2.00	3.00	2.29	2.00	0.02

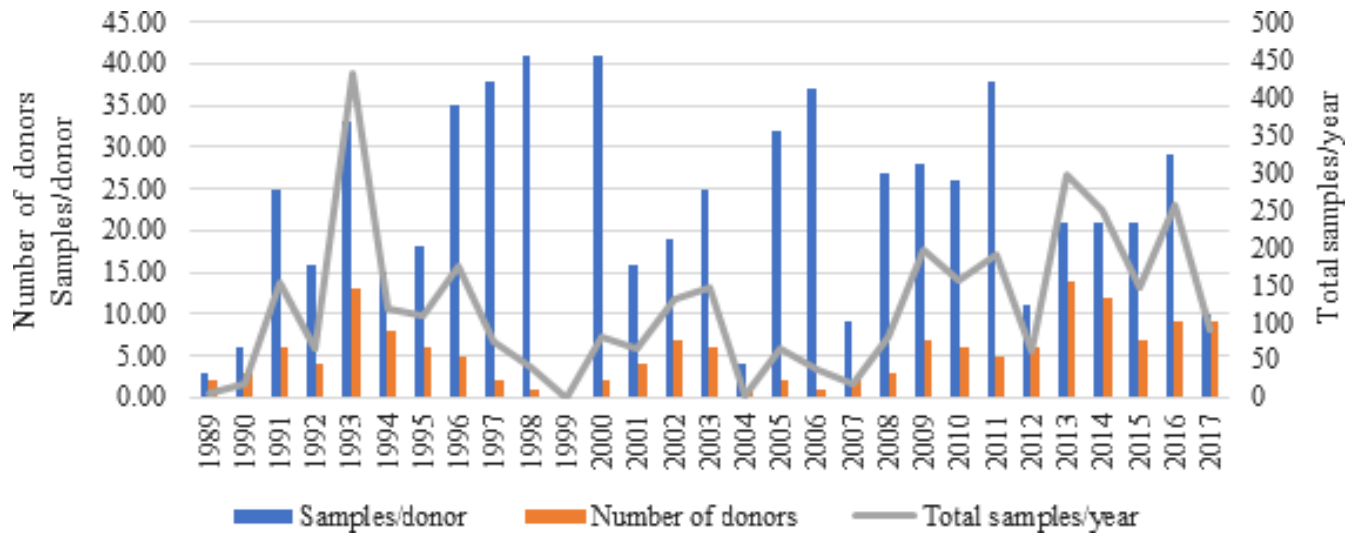
**FIGURE 2** Number of semen samples donated per individual donor

physical characteristics, ancestry origin and educational level. The results of the present study are in agreement with those of previous studies also reporting a decline in sperm concentration over the last few years. However, no differences were observed in total sperm number per ejaculate due to the increase in semen volume values, thus reflecting no real changes in sperm production over the years.

Donor sperm banking was formally established in Uruguay in 1989, when Fertilab inaugurated a heterologous sperm bank donor programme. From the onset, a strict screening protocol was followed to select potential sperm donors. The DAP that needs to be adhered to include the successful passing of a medical history, psychophysical examination and various laboratory-based tests and analysis (e.g. genetics and infective disease screening). Once a donor is accepted, semen samples are collected weekly or biweekly. During the study period, seventy-one (71) males voluntarily donated 3,449 sperm samples that were banked at Fertilab.

In March 2015, a statute concerning gamete donation was published in Uruguay. According to regulations in the statute, the act of gamete donation is one of altruism and confidentiality; therefore, the anonymity of the donor's identity must be warranted (Regulaciones de las técnicas de reproducción asistida 2015). Furthermore, it was also decided to limit the number of offspring per donor to 25 in order to minimise the risk of accidental consanguinity. From experiences in our laboratory, it is estimated that it takes on average more or less

40 donations per donor to achieve 10 ongoing pregnancies (Fertilab, 2016). According to our results, on average each donor banked about  $49.56 \pm 3.96 \approx 50$  samples, corresponding to approximately 12.5 pregnancies per donor during the study period. This valuation is based on ongoing pregnancy rates and does not consider delivery rate, thereby keeping us well within the limit of 25 children born per donor. Since the regulation was approved in 2015 in Uruguay, tracking of delivery rate per donor was protocolised and strictly followed in our institution. From all the information collected to date, it is apparent that none of our donors fathered more than 25 children. The American Society for Reproductive Medicine (ASRM) recommends a limit of 25 children per population of 800,000 for a single donor (Practice Committee of American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology, 2013). It is believed that this ratio will prevent any significant increased risk of inadvertent consanguineous conception. However, the ASRM's recommendations may require modification if the population using donor insemination represents an isolated subgroup or if the specimens are distributed over a wide geographic area (Practice Committee of American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology 2013). Considering that Fertilab is the only sperm bank in Uruguay and that the Uruguayan population is close to 3.4 million, our authorities should perhaps reevaluate the limit set on donor offspring. An appropriate example is New Zealand, where



**FIGURE 3** Annual number of donors, average of samples per donor and total samples over the 28-year period

**TABLE 3** Sperm parameter values of 3,449 donor samples from 1989 to 2017 clustered in subgroups

Variable	Number of Donors	Donations (n)	Year	P <sub>25</sub>	P <sub>75</sub>	Median	Mean	SG	SE	% Differences
Concentration ( $\times 10^6$ /ml)	24	882	1989–1995	70	120	90	93.24	a	1.25	-24.79
	14	567	1996–2002	70	120	95	95.39	a	1.48	
	16	548	2003–2009	73.75	110	90	92.62	a	1.75	
	24	1,452	2010–2017	47.75	98	74.8	74.72	b	0.90	
Volume (ml)	24	882	1989–1995	1.8	3	2.2	2.47	a	0.04	29.87
	14	567	1996–2002	2	3	2.5	2.49	a	0.03	
	16	548	2003–2009	2.1	3.725	3	3.16	b	0.06	
	24	1,452	2010–2017	2.4	4.5	3.3	3.53	c	0.04	
Total sperm count ( $\times 10^6$ )	24	882	1989–1995	135	286	200	223.27	a	4.10	9.67
	14	567	1996–2002	160	300	220	235.81	a,b	4.79	
	16	548	2003–2009	180	350	260	287.59	c	7.08	
	24	1,452	2010–2017	152.45	329	216	247.15	b	3.53	
Total Motility (PR + NP, %)	24	882	1989–1995	66	76	71	70.85	a	0.34	1.35
	14	567	1996–2002	67	77	72	71.13	b	0.44	
	16	548	2003–2009	69	79	74	73.99	c	0.42	
	24	1,452	2010–2017	67	79	73	71.82	b	0.30	
Progressive motility (%)	24	882	1989–1995	57	68	63	62.11	a	0.36	6.86
	14	567	1996–2002	58	68	64	62.05	a	0.47	
	16	548	2003–2009	61	73	66	66.27	b	0.41	
	24	1,452	2010–2017	61	74	68	66.69	b	0.32	

Note: Different letters within the SG column denote significant differences,  $p < .05$  according to Dunn's multiple comparisons test.

% Differences are calculated between means of the first (1989–1995) and the last period (2010–2017).

Abbreviations: SG, significant differences; SE, standard error.

a limit of ten children per donor, spread across four families, has been set (Gong et al., 2009). This rule is highly appropriate and should probably be adopted in Uruguay considering that New Zealand's population is similar to that of Uruguay.

Physical characteristics among donors are in line with the Uruguayan ethnography. According to the Continuous Reporting

System on International Migration in the Americas (SICREMI) (Organization of American States migration reporting system), sixty per cent (60%) of residents in Montevideo, Uruguay, were foreigners in 1843 and these immigrants were pre-dominantly from Italian and Spanish origins. Similarly, 74.65% of the donor population trace their origin to Europe, with the two countries

**TABLE 4** Sperm parameter values according to number of samples banked per donor

Samples/donor	Number of donors	Concentration ( $\times 10^6$ /ml)	Volume (ml)	Total motile spermatozoa ( $\times 10^6$ )	Total motility (PR + NP, %)	Progressive motility (%)
≤50	43	82.951 ± 1.065 <sup>a</sup>	3.241 ± 0.044 <sup>a</sup>	177.165 ± 3.1583 <sup>a</sup>	71.291 ± 0.327 <sup>a</sup>	62.831 ± 0.325 <sup>a</sup>
50–100	21	89.145 ± 1.044 <sup>b</sup>	2.604 ± 0.029 <sup>b</sup>	174.286 ± 2.409 <sup>a</sup>	72.826 ± 0.299 <sup>b</sup>	66.773 ± 0.327 <sup>b</sup>
≥100	7	81.927 ± 1.160 <sup>a</sup>	3.468 ± 0.054 <sup>c</sup>	191.252 ± 3.712 <sup>b</sup>	71.409 ± 0.324 <sup>a</sup>	64.119 ± 0.340 <sup>a</sup>

Note: Different superscripts within same column denote significant differences  $p < .05$  by Dunn's multiple comparisons test.

Values are expressed as Mean ± SE

mentioned previously topping the list. Caucasian accordingly describes the majority of the donor population as our results indicate pale skin tone, light brown eyes and brown hair to be the most common and main features.

The majority of donors were enrolled in college (51.4%). In 2016, Freeman et al. performed an online survey on 383 men registered as sperm donors with Pride Angel, a large UK-based connection website for donors and recipients of sperm. Interestingly, <1.30% of the study population represented Latin American countries (Freeman, Jadvá, Tranfield, & Golombok, 2016). Despite this, our results were highly comparable to this survey's as the majority (59.0%) of their

donors had a university degree followed by 30% with a college degree. This means that the results of this study are very similar to that observed in developed countries.

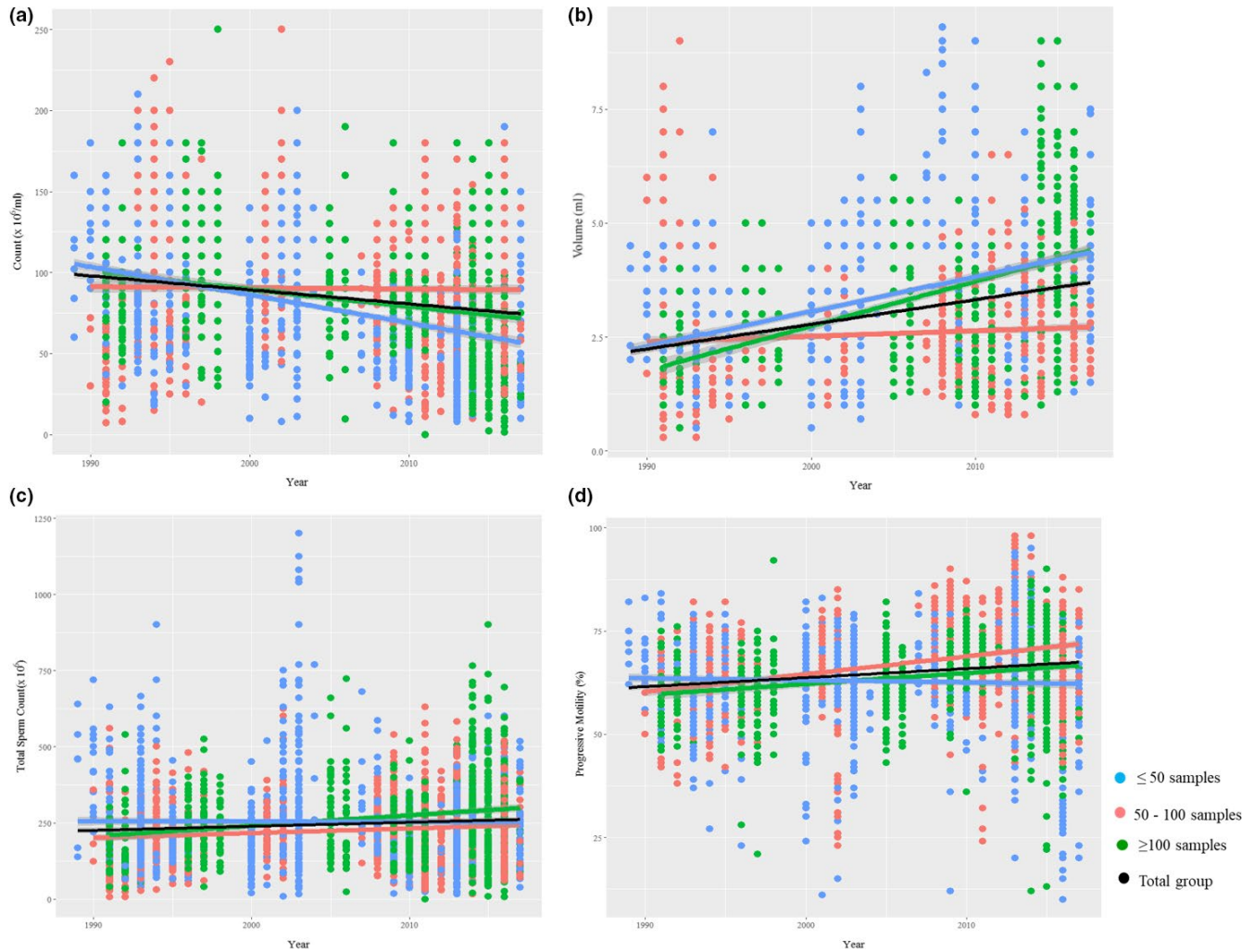
According to the cryotolerance test performed on the donors' first sample (Table 1), the recovery rate for progressively motile spermatozoa was 54%, which is in agreement with published data showing that on average 50% of motile sperm survives the cryopreservation process (World Health Organization, 2010). However, some authors reported only a 20% loss of total motility for donor samples (Matorras & Hernández, 2007). It is common to find a reduction of up to 50% in viable cells after cryopreservation, even in fertile men (Vieira et al., 2012). Our post-thaw viability recovery rate (76%) is in agreement with values expected and reported in the literature (Counsel, Bellinge, & Burton, 2004; Ordoqui et al., 2018; Verheyen et al., 1993; World Health Organization, 2010). Upon analysing the total number of samples banked over the 28-year period, the results showed that the median of the sperm values were normal according to the WHO 2010 lower reference values (World Health Organization, 2010) (Table 2).

To evaluate possible differences/changes in semen quality of the Fertilib donors from 1989 to March 2017, the samples were clustered and divided into four subgroups. Analysis revealed a nearly 24.77% decrease in sperm concentration over time when comparing the 1989–1995 to the 2010–2017 period. These results are in agreement with those of other studies that also showed a decrease in sperm concentration (Merzenich et al., 2010; Sengupta, Borges, et al., 2017). However, many findings are contradictory as some authors have not found a decline in concentration or ejaculate volume (Benshushan et al., 1997). An increase of nearly 4.19% in ejaculated semen volume was observed in-donor population during the 28-year period. This observation is supported by previous reports as a retrospective analysis, performed on 188 donor samples examined over a 15-year period in Jerusalem, found a 0.1 ml (5.10%) augmentation per year (Jorgensen, 2001). Another study (Paulsen, Berman, & Wang, 1996) also described a slight increase in the main sperm parameters (concentration, semen volume, total number of spermatozoa and normal morphology). However, it is well accepted that regional differences can be found in sperm quality, as is the case for variations observed among European countries (Auger & Jouannet, 1997; Iwamoto et al., 2006; Jorgensen, 2001). However, there are few, if any, longitudinal studies that evaluate sperm characteristics in healthy young Latin American donors. Therefore, this type of study could better mirror the impact of lifestyle, socioeconomics and the

**TABLE 5** Correlation values between semen parameters and year of donation according to number of samples banked and total group

Parameters	Year	
	( $\rho$ )	$p$ value
≤50 samples		
Concentration ( $\times 10^6$ /ml)	-0.41	<.001
Volume (ml)	0.459	<.001
Total Sperm Count	0.002	.928
Progressive Motility (%)	0.014	.601
50–100 samples		
Concentration ( $\times 10^6$ /ml)	-0.017	.5087
Volume (ml)	0.166	<.001
Total Sperm Count	0.108	<.001
Progressive Motility (%)	0.327	<.001
≥100 samples		
Concentration ( $\times 10^6$ /ml)	-0.33	<.001
Volume (ml)	0.598	<.001
Total Sperm Count	0.162	<.001
Progressive Motility (%)	0.191	<.001
Total Group		
Concentration ( $\times 10^6$ /ml)	-0.212	<0.001
Volume (ml)	0.359	<.001
Total Sperm Count	0.101	<.001
Progressive Motility (%)	0.197	<.001

Note: Spearman's Correlation.  $\rho$ , correlation coefficient. Correlation is significant,  $p \leq .05$ .



**FIGURE 4** Correlation between concentration ( $\times 10^6/\text{ml}$ ) (a), volume (ml) (b), total sperm count ( $\times 10^6$ ) (c), percentage of progressive motility (d) and year of donation. Groups were divided according to number of samples banked per donor, each dot represents one variable value, lines indicate linear trend among groups and total group

environment. One retrospective study performed between 1992 and 2003 in Brazil evaluated and compared the first and second samples banked by donors. A total of 182 donors were examined, and the results suggested that sperm concentration decreased significantly over the years. However, in line with our observations, ejaculate volume showed a slight increase. The decreasing trend in sperm concentration observed in our donor population, combined with the simultaneous increase in semen volume, and translated to significant increases in total sperm count (+9.66%). The slight augmentation in progressive motility (+6.86%) in combination with the trends observed for volume and concentration translated to an increase in total motile spermatozoa (+14.66) (Table 3). The analysis of the data using this approach did not consider similar number of samples over the years between groups. It was therefore decided to further cluster and divide it into three subgroups based on similar number of samples donated (Table 4). The same trend was observed for each studied variable. These results furthermore confirmed our observation regarding the longitudinal decline in sperm concentration and increase in semen volume over time.

Overall, our results showed a decline in sperm concentration and an increase in semen volume over time, while total sperm count remains unchanged. This observation allows us to conclude that testicular production did not show variations over the period of the study. Sengupta, Borges, et al. (2017) reviewed several studies conducted globally from 1980 to 2015. The data identified an overall diminution in sperm concentration over a span of 35 years, similar to our results where a significant decline in the sperm concentration over time was observed ( $r = -.212$ ,  $p < .001$ ). However, when the authors analysed the data according to geographical areas, no variations in sperm concentration were observed for the South America region ( $r = -.155$ ,  $p = .688$ ). The majority of the data were for men from Brazil and Argentina, and unfortunately, no data from Uruguay were included. The authors suggested that agricultural countries of South America are potentially more susceptible to exposure to endocrine disruptors, which might be more detrimental to men's fertility in the coming years (Sengupta, Dutta, & Krajewska-kulak, 2017). Therefore, further studies need to be performed to identify causes and explain the possible variations in sperm parameters

over time. In general, it is believed that environment and lifestyle are the most deleterious agents affecting semen quality as well as the regional discrepancies. A global decrease in semen volume has not been demonstrated (Levine et al., 2017; Sengupta, 2015), while our results actually showed a significant increase ( $r = .359, p < .001$ ). However, it is generally believed that there is no real relationship between semen volume and pregnancy rates (Bonde et al., 1998; Bostofte, Serup, & Rebbe, 1982). This observed significant increase in volume contributes to a significant positive correlation and increase in total sperm count ( $r = .101, p < .001$ ) over time. Moreover, a slight significant correlation was also observed for progressive motility ( $r = 0.197, p < .001$ ). When these parameters were combined, the results revealed that total motile spermatozoa also increased (14.665%, Table 3) over time. We can therefore conclude that while some semen parameters (e.g. sperm concentration) appeared to be compromised, the overall quality of the semen was not compromised in our population over the nearly three decades.

This study on the entire Uruguayan semen donor population highlights that our donor profile and semen properties are highly comparable to global observations.

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## REFERENCES

- Auger, J., & Jouannet, P. (1997). Evidence for regional differences of semen quality among fertile French men. *Fédération Française des Centres d'Etude et de Conservation des Oeufs et du Sperme humains*. *Human Reproduction*, 12(4), 740–745. <https://doi.org/10.1093/humrep/12.4.740>
- Benshushan, A., Shoshani, O., Paltiel, O., Schenker, J. G., & Lewin, A. (1997). Is there really a decrease in sperm parameters among healthy young men? A survey of sperm donations during 15 years. *Journal of Assisted Reproduction and Genetics*, 14(6), 347–353. <https://doi.org/10.1007/BF02765840>
- Bonde, J. P. E., Ernst, E., Jensen, T. K., Hjollund, N. H. I., Kolstad, H., Scheike, T., ... Olsen, J. (1998). Relation between semen quality and fertility: A population-based study of 430 first-pregnancy planners. *The Lancet*, 352(9135), 1172–1177. [https://doi.org/10.1016/s0140-6736\(97\)10514-1](https://doi.org/10.1016/s0140-6736(97)10514-1)
- Bonde, J. P., Giwercman, A., & Ernst, E. (1996). Identifying environmental risk to male reproductive function by occupational sperm studies: Logistics and design options. *Occupational and Environmental Medicine*, 53(8), 511–519. <https://doi.org/10.1136/OEM.53.8.511>
- Bostofte, E., Serup, J., & Rebbe, H. (1982). Relation between sperm count and semen volume, and pregnancies obtained during a twenty-year follow-up period. *International Journal of Andrology*, 5(3), 267–275. <https://doi.org/10.1111/j.1365-2605.1982.tb00255.x>
- Cooper, T. G., Noonan, E., von Eckardstein, S., Auger, J., Baker, H. W. G., Behre, H. M., ... Vogelsohn, K. M. (2009). World Health Organization reference values for human semen characteristics. *Human Reproduction Update*, 16(3), 231–245. <https://doi.org/10.1093/humupd/dmp048>
- Counsel, M., Bellinge, R., & Burton, P. (2004). Vitality of oligozoospermic semen samples is improved by both swim-up and density gradient centrifugation before cryopreservation. *Journal of Assisted Reproduction and Genetics*, 21(5), 137–142. <https://doi.org/10.1023/B:JARG.0000031245.39921.2c>
- Fertilab (2016). *Banco de gametos*. Retrieved from <http://www.bancodegametos.com.uy/pc/index.php#>
- Freeman, T., Jadvá, V., Tranfield, E., & Golombok, S. (2016). Online sperm donation: A survey of the demographic characteristics, motivations, preferences and experiences of sperm donors on a connection website. *Human Reproduction*, 31(9), 2082–2089. <https://doi.org/10.1093/humrep/dew166>
- Glina, S., Nova, T., Brand, V. B. F., Molina, E., Galuppo, A. G., Correa, N. R., & Moreira, F. R. (2010). Evaluation of semen parameters in semen donors in a ten-year period in the city of São Paulo. *Einstein*, 8(4), 423–429. <https://doi.org/10.1590/S1679-45082010AO1546>
- Gong, D., Liu, Y.-L., Zheng, Z., Tian, Y.-F., Li, Z., & Zheng, L. (2009). An overview on ethical issues about sperm donation. *Asian Journal of Andrology*, 11(11), 645–652. <https://doi.org/10.1038/aja.2009.61>
- Homan, G. F., Davies, M., & Norman, R. (2007). The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: A review. *Human Reproduction Update*, 13(3), 209–223. <https://doi.org/10.1093/humupd/dml056>
- Iwamoto, T., Nozawa, S., Yoshiike, M., Hoshino, T., Baba, K., Matsushita, T., ... Jørgensen, N. (2006). Semen quality of 324 fertile Japanese men. *Human Reproduction*, 21(3), 760–765. <https://doi.org/10.1093/humrep/dei362>
- Jensen, T. K., Slama, R., Ducot, B., Suominen, J., Cawood, E., Andersen, A. G., ... Spira, A. (2001). Regional differences in waiting time to pregnancy among fertile couples from four European cities. *Human Reproduction*, 16(12), 2697–2704. <https://doi.org/10.1093/humrep/16.12.2697>
- Jørgensen, N., Andersen, A.-G., Eustache, F., Irvine, D. S., Suominen, J., Petersen, J. H., ... Skakkebaek, N. E. (2001). Regional differences in semen quality in Europe. *Human Reproduction*, 16(5), 1012–1019. <https://doi.org/10.1093/humrep/16.5.1012>
- Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Mindlis, I., ... Swan, S. H. (2017). Temporal trends in sperm count: A systematic review and meta-regression analysis. *Human Reproduction Update*, 23(6), 646–659. <https://doi.org/10.1093/humupd/dmx022>
- Macleod, J., & Gold, R. Z. (1951). The male factor in fertility and infertility. II. Spermatozoon counts in 1000 men of known fertility and in 1000 cases of infertile marriage. *The Journal of Urology*, 66(3), 436–449.
- Marimuthu, P., Kapilashrami, M. C., Misro, M. M., & Singh, G. (2003). Evaluation of trend in semen analysis for 11 years in subjects attending a fertility clinic in India. *Asian Journal of Andrology*, 5(3), 221–225. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12937806>
- Matorras, R., & Hernández, J. (2007). *Estudio y tratamiento de la pareja estéril: Recomendaciones de la Sociedad Española de Fertilidad, con la colaboración de la Asociación Española para el Estudio de la Biología de la Reproducción, de la Asociación Española de Andrología y de la Sociedad Es. Adalia*. Madrid.
- Mendiola, J., Torres-cantero, A. M., & Agarwal, A. (2009). Lifestyle factors and male infertility: An evidence-based review. *Archives of Medical Sciences*, 5(1A), S3–S12.
- Merzenich, H., Zeeb, H., Blettner, M., Carlsen, E., Giwercman, A., Keiding, N., ... Jørgensen, N. (2010). Decreasing sperm quality: A global problem? *BMC Public Health*, 10(1), 24. <https://doi.org/10.1186/1471-2458-10-24>
- Nallella, K. P., Sharma, R. K., Aziz, N., & Agarwal, A. (2006). Significance of sperm characteristics in the evaluation of male infertility. *Fertility and Sterility*, 85(3), 629–634. <https://doi.org/10.1016/j.fertnstert.2005.08.024>

- Ordoqui, R., Barrera, N., Montes, J. M., Canepa, M., Bonelli, C., Surka, C., ... Plessis, S. S. (2018). A retrospective study on sperm banking: A Uruguayan experience. *JBRA Assisted Reproduction*, 22(2), 82–88. <https://doi.org/10.5935/1518-0557.20180037>
- Paulsen, C. A., Berman, N. G., & Wang, C. (1996). Data from men in greater Seattle area reveals no downward trend in semen quality: Further evidence that deterioration of semen quality is not geographically uniform. *Fertility and Sterility*, 65(5), 1015–1020. [https://doi.org/10.1016/S0015-0282\(16\)58279-X](https://doi.org/10.1016/S0015-0282(16)58279-X)
- Practice Committee of American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology (2013). Recommendations for gamete and embryo donation: A committee opinion. *Fertility and Sterility*, 99(1), 47–62.e1. <https://doi.org/10.1016/j.fertnstert.2012.09.037>
- Regulaciones de las técnicas de reproducción asistida, Pub. L. No. N° 31/014, 2088 (2015). *Uruguay*. Retrieved from , <https://www.impo.com.uy/bases/decretos/311-2014https://www.impo.com.uy/bases/leyes/19167-2013>
- Sandler, B. (1953). The male factor in human sterility. *The Journal of Obstetrics and Gynaecology of the British Empire*, 60(1), 67–75. <https://doi.org/10.1111/j.1471-0528.1953.tb06414.x>
- Sengupta, P. (2015). Reviewing reports of semen volume and male aging of last 33 years: From 1980 through 2013. *Asian Pacific Journal of Reproduction*, 4(3), 242–246. <https://doi.org/10.1016/j.apjr.2015.06.010>
- Sengupta, P., Borges, E., Dutta, S., & Krajewska-Kulak, E. (2017). Decline in sperm count in European men during the past 50 years. *Human & Experimental Toxicology*, 37(3), 247–255. <https://doi.org/10.1177/0960327117703690>
- Sengupta, P., Dutta, S., & Krajewska-kulak, E. (2017). *The Disappearing Sperms: Analysis of Reports Published Between 1980 and 2015*. Retrieved from <https://doi.org/10.1177/1557988316643383>
- Van der Horst, G., & du Plessis, S. S. (2017). Not just the marriage of Figaro: But the the marriage of WHO / ESHRE semen analysis criteria with sperm functionality. *Advances in Andrology*, 4(2010), 6–21.
- Verheyen, G., Pletincx, I., & Van Steirteghem, A. (1993). Andrology: Effect of freezing method, thawing temperature and post-thaw dilution/washing on motility (CASA) and morphology characteristics of high-quality human sperm. *Human Reproduction*, 8(10), 1678–1684. <https://doi.org/10.1093/oxfordjournals.humrep.a137912>
- Vieira, M. M., Nery, S. F., Tavares, R. L., Cruz, C. D., Reis, F. M., & Camargos, A. F. (2012). Rapid thawing human sperm does not affect basic parameters in normozoospermic men: A double-blind prospective study. *International Brazilian Journal of Urology*, 38(1), 108–115. <https://doi.org/10.1590/S1677-55382012000100015>
- Willem, O., Ox, A. C., & Anssen, J. (2009). In search for the general population' s semen profile: The study of sperm parameters in partners of women with chronic anovulation. *Facts, Views & Vision in ObGyn*, 1(1), 18–26.
- World Health Organization (2010). *WHO laboratory manual for the examination and processing of human semen* (5th ed). Geneva: World Health Organization.

### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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