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The Effect of Age, Sperm Freezing, and Washing on Intrauterine Insemination Outcome: a Retrospective Analysis

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Abstract

Age and semen quality can significantly affect the outcome of intrauterine insemination treatment. However, few studies have evaluated the effect of age, semen cryopreservation, and washing on intrauterine insemination outcomes. The current study evaluates the effects of woman age and semen donor age, semen processing, and freezing on intrauterine insemination outcomes. Significant negative correlations were found between semen donors' age and sperm concentration, progressive motility, and normal morphology. Donors aged less than or equal to 30 years had better semen quality compared to those aged above 30 years. Significant higher semen viscosity, semen volume, total sperm count, progressive motility, total progressively motile count, normal morphology, and total normal sperm count were observed in fresh semen samples of donors who had positive pregnancies after intrauterine insemination. Furthermore, significantly higher post-wash progressive motility was obtained in donors who had positive pregnancies after intrauterine insemination. The results of this study provide insight into the eligibility in terms of age and semen characteristics of patients seeking intrauterine insemination.

Keywords: Aging, Artificial Insemination, Cryopreservation, Semen Quality

Introduction

The global prevalence of infertility was reported to be approximately 15% in 2015 (1). Different assisted reproductive techniques are used to treat infertility. Intrauterine insemination (IUI) is the ART consisting of placing washed semen samples in the upper uterine cavity (2). IUI is considered the first line (3, 4) and cost-effective (5, 6) treatment. IUI is frequently used for mild male factor infertility, anovulation, endometriosis, and unexplained infertility (7, 8).

Several factors can influence IUI outcome, which includes maternal age (9-11), paternal age (12, 13), and semen parameters (14-17). Maternal age significantly influenced clinical pregnancy rate and live birth rates after intrauterine insemination after controlled ovarian stimulation (18). Advanced female age was previously indicated to harm the pregnancy rate and associated with an increased miscarriage rate (19). Although the effect of paternal age on IUI outcome is not yet established in the literature (20), an increased number of spermatozoa with necrospermia and DNA fragmentation due to advanced paternal age were found to negatively impact intrauterine insemination outcomes (19).

Many IUI cycles are using frozen semen samples. Cryopreservation is a core art technique that can provide long-term preservation of spermatozoa, enabling the conservation of male fertility (21). The process of freezing spermatozoa has been used routinely for over 40 years to preserve fertility ability in males undergoing cancer therapy (22), medical surgery that may reduce the testicular function or induce sexual dysfunction, or in male facing autoimmune diseases and those seeking vasectomy surgery (23-27). Furthermore, the freezing of spermatozoa is used in clinical settings by sperm banks for infertility treatment and the establishment of sperm donors (28). However, freezing of spermatozoa was found to reduce sperm viability (29), normal morphology (30) total motile sperm count (31), and induce sperm DNA damage (32). There is a notable lack of studies investigating the association between human semen freezing and IUI outcome. The current study evaluates the effects of age, semen processing, and freezing on IUI outcomes.

Materials and methods

We retrospectively analyzed 40 IUI cycles using frozen donor semen samples between June 2017 and November 2022 at Androcryos andrology laboratory, south Africa. Demographic data such as woman and donor age together with fresh, post-freeze, and post-wash semen parameters were analyzed. Semen samples were collected and evaluated according to the methodology described by the world health organization (who, 2010). Semen parameters evaluated in the current study consisted of viscosity (mm), volume (ml), sperm concentration ($x10^6$/ml), total sperm count ($x10^6$), progressive motility (%), total progressive motile count (%), normal morphology (%), total normal sperm count (%).

The total progressive motile count was defined as the product

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of total sperm count and progressive motility (sperm concentration (106 /ml)/100 x progressive motility (%) x volume (ml)), while the total normal sperm count was the product of total sperm count and the percentage of normal morphology (sperm concentration (106 /ml)/100 x normal morphology (%) x volume (ml)).

Permission to use the data was granted by the androcryos andrology laboratory director.IUI was performed under gonadotropin stimulation. Fresh semen samples collected from donors were initially evaluated, then frozen using spermfreeze^{tm} (fertipro, (fertipro, Brussels, Belgium). Semen samples were processed using a density gradient (puresperm®, nidacon international ab, Sweden) and sperm wash medium (sperm wash, nidacon, international ab, Sweden).

Statistical analysis was performed using the medcalc® statistical software version 19.5 (medcalc software ltd, Ostend, Belgium; https:www.medcalc.org; 2020). Descriptive statistics for variables were presented as sample size, minimum, maximum, mean, median, and standard deviation. The chi-square test was used to determine the distribution of all the data sets. Correlations were determined by Pearson correlation and expressed as r^2. For all statistical tests, a p-value of < 0.05 was considered statistically significant. All data collection and statistical analyses were done by the author (lw).

Results

Summary Statistics

Summary statistics of the age and evaluated fresh semen parameters are summarized in Table 1. The average woman age and donor age in this study were 33 ±4.07 and 29 ±3.05 respectively. The average semen and volume were 3.79 ±1.28 and 27.55 ±23.53 respectively. Sperm concentration was 103.2 ±30.77 while total sperm count was 389.19 ±165.82. The average donor sperm progressive motility and total progressive motile count were 68.50 ±11.83 and 277.60 ±151.97. Normal morphology was 7.20 ±2.90 while the total normal sperm count was 30.68 ±22.23.

Correlation between donor age and semen parameters

Table 2 summarizes the correlation coefficient and significance between donor age and semen parameters. Significant negative correlations were found between donor age and sperm concentration, progressive motility, and normal morphology ($p<0.05$). Significant positive correlation between semen viscosity and progressive motility, normal morphology, and between semen volume and normal morphology. Furthermore, there was a significant positive correlation between sperm concentration and normal morphology as well as between progressive motility and normal morphology.

Comparison of pre-freeze, post-freeze, and post-wash semen parameters between donors aged less than or equal to 30 years and more than 30 years

The comparison of semen parameters between donor age groups (≤30 years and >30 years) is highlighted in Table 3. Donors aged less than or equal to 30 years had significantly higher total sperm count, progressive motility, normal morphology, and total normal sperm count ($p<0.05$).

Impact of age and fresh semen parameters on pregnancy outcomes

A comparison between the age of women who had positive pregnancies and those who had negative pregnancies is highlighted in Table 4. Furthermore, the table summarizes the comparison of donor age and semen characteristics between the positive pregnancy group (group i) and the negative pregnancy group (group ii). There were no significant differences in woman and donor age between the group of women who had positive pregnancies and those who had negative pregnancies. However, semen viscosity, semen volume, total sperm count, progressive motility, tpmc, normal morphology, and tnsc of the donor in the positive pregnancy group were significantly higher than those in the negative pregnancy group. Sperm concentration was not significantly different between the 2 groups of donors.

Comparison Of Post-Freeze And Post-Wash Semen Parameters

Table 5 summarizes the comparison of post-freeze and post-wash sperm concentration and progressive motility between donors' sperm with positive pregnancy (group i) and donor sperm with negative pregnancy (group ii) outcomes. There were no significant differences in post-freeze concentration, post-freeze progressive motility, and post-wash concentration between group 1 and group 2. However, group I had a significantly higher post-wash progressive motility ($p=0.0060$) in comparison to group ii.

Table 1. Summary statistics of the age and fresh semen parameters obtained from participants

	N	Minimum	Maximum	Mean	Median	SD	Distribution
Women Age	40	25	41	33.77	33.00	4.07	Normal
Donor Age	40	26	35	29.57	28.50	3.05	Normal
Viscosity	40	2.00	100	27.55	22.50	23.53	Normal
Volume	40	2.20	6.50	3.79	3.30	1.28	Normal
Concentration	40	60	178	103.2	100	30.77	Normal
Total Sperm Count	40	142.60	747.60	389.19	337.25	165.82	Normal
Progressive Motility	40	50.00	95.00	68.50	65.00	11.83	Normal
Total Progr. Motile Count	40	71.30	662.40	277.60	221.71	151.97	Normal
Normal Morphology	40	2.00	12.00	7.20	6.50	2.90	Normal
Total Normal Sperm Count	40	5.58	80.90	30.68	22.42	22.23	Normal

Table 2. The correlation coefficient between donor age and fresh semen parameters using the Pearson correlation coefficient

		Viscosity	Semen Volume	Sperm Concentration	Progressive Motility	Normal Morphology
Donor Age	**R^2**	-0,262	-0,206	-0,358	-0,391	-0,395
	P	0,1028	0,2021	0,0233	0,0127	0,0116
	N	40	40	40	40	40
Viscosity	**R^2**		0,183	0,070	0,330	0,452
	P		0,2588	0,6667	0,0376	0,0034
	N		40	40	40	40
Semen Volume	**R^2**			-0,064	0,222	0,452
	P			0,6968	0,1690	0,0034
	N			40	40	40
Sperm Concentration	**R^2**				0,529	0,311
	P				0,0005	0,0512
	N				40	40
Progressive Motility	**R^2**					0,375
	P					0,0171
	N					40

Table 3. Comparison of pre-freeze, post-freeze, and post wash semen parameters between donor aged less than or equal to 30 years and more than 30 years

	Donor Age ≤30		Donor Age >30		P-Value
	N	**Mean (±SD)**	**N**	**Mean (±SD)**	
Semen Viscosity	26	31.88 (±25.26)	14	21.42 (±19.15)	0.187
Semen Volume	26	4.04 (±1.40)	14	3.40 (±1.00)	0.143
Sperm Concentration	26	109.76 (±28.88)	14	93.85 (±32.23)	0.122
Total Sperm Count	26	435.59 (±160)	14	329.12 (±151.70)	0.033
Progressive Motility	26	71.800 (±11.71)	14	62.85 (±10.50)	0.023
TPMSC	26	320.02 (±143.8)	14	211.91 (±146.36)	0.031
Normal Morphology	26	8.04 (±2.96)	14	5.92 (±2.26)	0.026
Total Normal Sperm Count	26	36.97 (±21.99)	14	21.04 (±19.38)	0.029

Table 4. Comparison of age and fresh semen parameters between positive and negative pregnancy outcomes groups

		Positive Pregnancy (Group I)	Negative Pregnancy (Group II)	P-Value
Woman Age	**Mean (±SD)**	32.57 (±3.33)	34.85 (±4.44)	0.076
	N	19	21	
Donor Age	**Mean (±SD)**	28.73 (±2.82)	30.33 (±3.11)	0.099
	N	19	21	
Semen Viscosity	**Mean (±SD)**	40.00 (±22.60)	16.28 (±18.34)	0.0008
	N	19	21	
Semen Volume	**Mean (±SD)**	4.44 (±1.15)	3.20 (±1.12)	0.0014
	N	19	21	
Concentration	**Mean (±SD)**	110.26 (±31.09)	96.80 (±29.76)	0.170
	N	19	21	
TSC	**Mean (±SD)**	479.32 (±150.50)	307.64 (±136.13)	0.0005
	N	19	21	
Prog. Motility	**Mean (±SD)**	72.89 (±13.97)	64.52 (±7.89)	0.023
	N	19	21	
TPMC	**Mean (±SD)**	358.68 (±155.05)	204.24 (±107.24)	0.0007
	N	19	21	
Norm. Morphology	**Mean (±SD)**	9.78 (±1.81)	4.85 (±1.15)	<0.0001
	N	19	21	
TNSC	**Mean (±SD)**	48.30 (±19.90)	14.74 (±6.58)	<0.0001
	N	19	21	

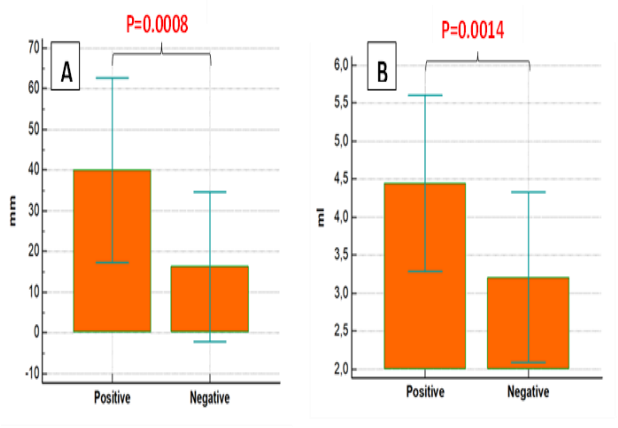

Figure 1. Illustration of the pre freeze semen viscosity (a) and semen volume (b), between positive pregnancy and negative pregnancy.

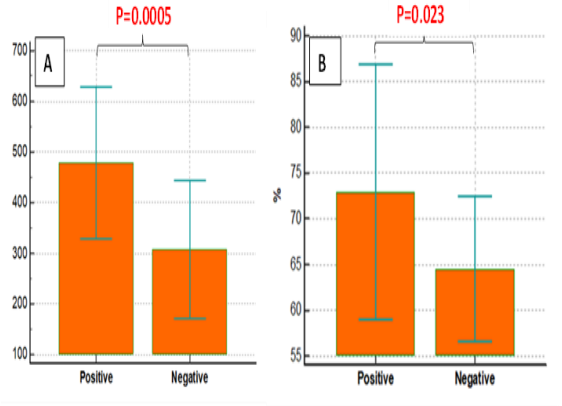

Figure 2. Illustration of the pre freeze total sperm count (a) and progressive motility (b), between positive pregnancy and negative pregnancy.

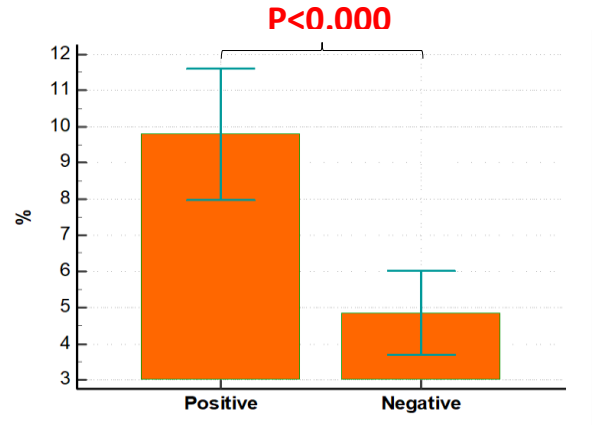

Figure 3. Illustration of the pre freeze normal morphology between positive pregnancy and negative pregnancy.

Table 5. Comparison of post-freeze and post-wash sperm concentration and progressive motility between positive and negative pregnancy outcomes groups

		Positive Pregnancy (Group I)	Negative Pregnancy (Group II)	P-Value
Post-Freeze Concentration	**Mean**	59.73	50.57	0.191
	SD	28.41	13.10	
	N	19	21	
Post-Freeze Prog Motility	**Mean**	51,0526	46,61	0.095
	SD	9.88	6.46	
	N	19	21	
Post-Wash Concentration	**Mean**	26.88	23.19	0.450
	SD	14.15	11.21	
	N	9	21	
Post Wash Prog. Motility	**Mean**	92.22	77.38	0.0060
	SD	2.63	14.71	
	N	9	21	

Discussion

Several factors were found to affect IUI outcomes (8). Advancing paternal age was associated with a decrease in semen quality (33-35), consequently negatively impacting pregnancy rates after IUI (8). An age-related decrease in sperm concentration and progressive motility was highlighted in a retrospective cross-sectional study investigating 71,623 infertile men in China (36). Furthermore, in a meta-analysis of 90 studies and 93,839 males, age-related declines are found for semen volume, progressive motility, total motility, normal morphology, and unfragmented cells, independent of confounding variables (37). This is reflected in the results of this retrospective study. Sperm concentration, progressive motility, and normal morphology had a significant negative correlation with donor age. Additionally, the current study found a significant positive correlation between sperm morphology and semen viscosity, semen volume, and progressive motility.

While some research has highlighted a decrease in semen quality associated with advancing paternal age, other studies have established an age threshold for semen parameters (35-37). A threshold of > 45 years for sperm concentration and motility decrease was identified in a retrospective analysis involving 889 men (38). More interestingly, some reports highlighted age thresholds > 35 years (35,36,39). For instance, Pino et al., 2020 found that males aged <31 years were more likely to experience a decrease in sperm motility (39). The current study has demonstrated that donors aged less than or equal to 30 years have significantly higher total sperm count, progressive motility, tpmc, normal morphology, and total normal sperm count than those aged above 30.

Semen parameters were indicated to have a predictive value for pregnancy outcomes in intrauterine insemination cycles (40, 41). Sperm progressive motility (42-44), total sperm count (42), normal morphology (40, 44, 45), and total motile sperm count (40;41) were found to be useful prognostic factors of IUI. This is consistent with the results of the current study which showed significantly higher progressive motility, total progressively motile count, total sperm count, normal morphology, and total normal sperm count in the positive pregnancy group compared to the negative pregnancy group. Furthermore, the current study also found significant differences in terms of semen volume and semen viscosity, which has not been previously reported to our knowledge. Surprisingly, the current study did not find any

difference in sperm concentration between the evaluated groups while previous studies have highlighted sperm concentration as an important predictive factor for IUI (42). However, it is important to note that the average sperm concentration for both investigated groups in the current study was above lower reference limits as stipulated in the WHO, 2010.

Sperm cryopreservation has been widely used in assisted reproductive technology (art) (49). Freezing of spermatozoa has been the best option to preserve male fertility before oncologic treatments (50, 51), for male patients facing severe oligospermia or ejaculatory disorder (49). A previous study has found that intrauterine insemination using frozen sperm resulted in an increase in the pregnancy rate in donor insemination cycles under gonadotropin stimulation (49). The current study did not find any differences between the two investigated groups in terms of post-freeze concentration, post-freeze progressive motility, and post-wash concentration. Although the current study didn't find any significant differences in post-wash sperm concentration between the two groups, both groups had post-wash sperm concentrations above the threshold value of 1 million for successful IUI which was previously observed in many studies (50-52). Similarly, to the fresh semen samples, significant differences were observed in post-wash progressive motility.

The predictive value of paternal age and semen parameters for pregnancy after the first IUI cycle is still controversial in the literature, with some studies showing low or no predictive power of some semen parameters (44;53). Our study showed significant negative correlations between donor age and semen parameters with better semen quality observed in donors aged less than or equal to 30 years. Furthermore, the semen quality of donors who had positive pregnancies following IUI was higher than donors who had negative pregnancies. Additionally, the current study found differences in post-wash progressive motility between the investigated groups. However, these conclusions should be interpreted with caution, a better multi-center prognostic study design including standardized analysis, consistent sperm processing methodology, and controlled fertility workup is needed to confirm the current results.

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Ethical Issue
The author is aware of and complies with, best practices in publication ethics specifically about authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. The author adheres to publication requirements that submitted work is original and has not been published elsewhere in any language. Also, all procedures performed in studies involving human participants were under the ethical standards of the institutional and/or national research committee and with the 1964 helsinki declaration and its later amendments or comparable ethical standards.

Competing Interests
The author declares that no conflict of interest would prejudice the impartiality of this scientific work.

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