

Effect of age on quality of semen parameters

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Abstract: Background: Increased life expectancy, advanced age of marriage, various socio-economic factors and an overall change in role of women in society has led couples to start their family at a later age. Higher maternal and paternal age raises the question of fertility maintenance during the ageing process. **Aim of the work:** to determine the relationship between male age and semen quality (semen volume, concentration, motility, and morphology) in healthy men. **Patients and methods:** This is a cross sectional comparative study that was carried out on a convenience sample of 452 apparently healthy male volunteers. Those volunteers were selected from (Kanater men's prison) in Cairo during the period from June 2016 to March 2017. They aged 20 – 60 years with no history of infertility or reproductive problems. All were submitted to full history taking, clinical examination with stress on genital examination and semen analysis. Semen parameters were evaluated following WHO standard criteria. Volunteers were grouped according to age into four groups as follow; 20-29, 30-39, 40-49 and ≥ 50 years. **Results:** Semen quality varied as regard age groups; It was found that the highest mean of seminal volume 3.01(± 0.8 SD) ml was recorded among men of age group 20-29 years and the lowest was among men of age group ≥ 50 years with mean 1.6 (± 0.9 SD) ml. As regard sperm concentration it was highest among those of age group 20-29 years with mean 92(± 24 SD) million/ml and the lowest was among men of age group ≥ 50 years with mean 79.8(± 13 SD) million/ml. Men of age group 20-29 years have the highest mean of total sperm count, progressive motility, non-progressive motility, total motility count and normal sperm morphology: 281.6 (± 114.5 SD) million, 33.3(± 6.8 SD) %, 17.9(± 4.5 SD) %, 145 (± 67.6 SD) million and 32.7 (± 3.4 SD) % respectively, Followed by those of age group 30-39 years and age group 40-49 years. While the lowest mean of these parameters were recorded among those of age group ≥ 50 years, they were as follows; 129 (± 42.5 SD) million, 17.7(± 8.1 SD) %, 10.8(± 4 SD) %, 38.8 (± 21 SD) million and 6.8 (± 2.1 SD) % respectively. All these differences were statistically significant. $p < 0.05$. There was significant negative correlation between age and semen volume, sperm concentration, total sperm count, progressive motility, non-progressive motility, total motility count and normal sperm morphology, while, positive correlation was found between age and immotility. **Conclusion:** significant age-related decreases in semen quality were observed, most notably for semen volume and sperm motility. Because semen quality is a proxy for fertility, these data suggest that men may become progressively less fertile as they grow older.

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1. Introduction

Increased life expectancy, advanced age of marriage, various socio-economic factors and an overall change in role of women in society has led couples to start their family at a later age (Bray et al., 2006). Higher maternal and paternal age raises the question of fertility maintenance during the ageing process (Levitas et al., 2007).

It has been established that aging in women significantly reduces the potential to produce oocytes and achieve conception. Female fecundity starts to decline after 30 years of age and is more pronounced after 40 years of age, thereby making maternal age the

main limiting factor in the treatment of infertility. However, very little data showed similar trends in men, possibly because of the fact that spermatogenesis can continue throughout life (Panayiotis et al., 2006).

Males have the advantage over females that they can contribute to conception even after the age of 40 and up to an age beyond 40 years of sexual maturity. However, in advanced ages, degenerative changes in germinal epithelium, decreased number of Leydig cells, and their functions affect spermatogenesis through decrease in testosterone level, starting at the age of 30 years (Sunanda et al., 2014). Furthermore, epidemiological evidence suggested that there was a

decline in semen quality (e.g., volume, motility, and morphology) and male fertility associated with increased male age (Silva et al., 2012).

Changes in the biochemistry of human semen have been reported that aging showed decreases in the concentrations of fructose, kallikrein and prostate specific antigen (PSA), and elevated liquefaction times. These alterations could cause age-related declines in sperm motility and fertilizing ability (Matsuda et al., 2004).

In addition, advanced paternal age has been implicated an increased frequency of miscarriages, autosomal dominant disorders and aneuploidies (Silva et al., 2012). Also it has been shown to be associated with numerous disorders like achondroplasia, autism, schizophrenia and bipolar disorders (D'Onofrio et al., 2014). 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 (La Rochebrochard and Thonneau, 2003; Molina et al., 2010).

The identification of aging helped to predict the age impact on the reproductive function. Furthermore, it will be possible to accurately establish an 'Age Threshold' which once crossed; a prospective father should attend a counseling session in which he should be educated about the risks involved with conceiving an offspring at old age. (Sharma et al., 2015).

The aim of this study is to determine the association between male age and semen quality (semen volume, concentration, motility, and morphology) in healthy men.

2. Patients and methods

Study design and sampling: This is a cross sectional comparative study that consisted of voluntary sample of 452 male volunteers from total 1023 at (Kanater men's prison) in Cairo, Egypt, it was used as the recruitment site because the place is relatively homogeneous. The study was carried out on apparently healthy men (volunteers) (aged 20 – 60 years) with no history of infertility or reproductive problems. They were divided into four groups according to their ages: 20-29, 30-39, 40-49 and ≥ 50 years. Then comparing between those ≤ 40 years and those > 40 years. Participants were selected during the period from June 2016 to march 2017. Patients with the following criteria were included in the study: non-smokers have semen samples with sperm concentrations of $\geq 15 \times 10^6 \text{ ml}^{-1}$.

Men with history of infertility problem, Men with history of lifestyle, diet, medical and occupational status that may affect semen quality and those with diabetes, infectious diseases, hydrocele, hernia, varicocele, addiction to tobacco or alcohol, over weight and obese were excluded from the study. Included volunteers were subjected to the following:

1- Full history taking (personal history, past history, sexual history, family history); 2- General examination, full genital examination that included penis, testes, epididymis, vas deferens, spermatic cord, scrotal swelling and inguinal examination and rectal examination; 3- Semen analysis; semen samples were collected by masturbation into wide mouth plastic container, in a room close to the andrology laboratory. The participants were advised to keep the abstinence period around 2-7 days. Samples were analyzed within 30-60 min, after liquefaction at 37°C . Semen parameters like sperm count/mL, percentage of motile spermatozoa, and percentage of normal spermatozoa were analyzed along with the presence of pus cells in semen according to the WHO standard criteria 2010.

About 6-10 μL of semen was put on to a microscopic slide and sperm motility was analyzed under bright field microscope under 400x magnification. At least 200 sperms were counted, and the mean value from duplicate measurements was represented. Sperm counts were done by using Neubauer's haemocytometer with requisite dilutions (1:2, 1:5, 1:20), as per the WHO manual, 2010. Diluted semen samples were mixed properly and 10 μL of the sample was transferred to Neubauer's chamber for counting of spermatozoa. Replicate dilutions were prepared to get correct values. Sperm morphology was assessed in Papanicolaou-stained smears (Haematoxyline, Orange-G and EA-50 stain) using light microscopy under oil immersion at 1000x magnification.

Vitality of sperms was estimated by the hypo-osmotic swelling (HOS) test that was performed by mixing equal volumes of semen and hypo-osmotic solution, prepared from 7.35 g sodium citrate and 13.5 g fructose in 1000 mL distilled water. The mixture was incubated for 30 min at 37°C , from which an aliquot of 10 μL was immediately examined at the 40x magnification. The percentage of swollen (vital) sperm was assessed by counting a minimum of 200 spermatozoa.

Duplicate samples were requested from donors if the sample exhibited any of the following: volume < 1 ml, zero motility, abnormal numbers of red or white blood cells, or potential loss of sample reported by donor.

Statistical design: The contrast of seminal parameter values (mean \pm SD) between four age groups was carried out using analysis of variance (ANOVA) test. Differences between those < 40 years and those ≥ 40 years were carried out using t -test. Correlation was done to examine the relationship between age of men and standard semen parameters. $P < 0.05$ was considered statistically significant. All the statistical analysis was performed with SPSS version 16.

Ethical consideration: Verbal consent was obtained from all participants. All samples were obtained and processed with the approval of the Ethics Committee, Faculty of Medicine, Al-Azhar University.

3. Results

The 452 men enrolled in the present study with on average 36 (± 10.1 SD) years old range 20-60 years. According to age group they were as follow; 20-29 (33.3%), 30-39 (38.5%), 40-49 (16.8%) and ≥ 50 (11.5%).

Semen parameters were as follows; mean of semen volume 2.7 (± 0.9 SD) ml, sperm concentration 89 (± 20 SD) million/ml, total sperm count mean 243.6 (± 110 SD) million, progressive motility 28.7 (± 8.1 SD)%, non-progressive motility 15.8 (± 4.7 SD)%, immotility 55.45 (± 11.2 SD)%, Total motility count (progressive + Non progressive) mean 114.2 (± 65.7 SD) million and normal sperm morphology 25 (± 9 SD) % (table 1). Semen quality varied as regard age groups; it was observed a statistically significant decrease in all semen parameters with increasing age except sperm immotility which increase with age. It was found that the highest mean of seminal volume 3.01 (± 0.8 SD) ml was recorded among men of age group 20-29 years and the lowest was among men of age group ≥ 50 years with mean 1.6 (± 0.9 SD) ml. As regard sperm concentration it was highest among those of age group 20-29 years with mean 92 (± 24 SD) million/ml and the lowest was among men of age group ≥ 50 years with mean 79.8 (± 13 SD) million/ml. Men of age group 20-29 years have the highest mean of total sperm count, progressive motility, non-progressive motility, total motility count and normal sperm morphology: 281.6 (± 114.5 SD) million, 33.3 (± 6.8 SD) %, 17.9 (± 4.5 SD) %, 145 (± 67.6 SD) million and 32.7 (± 3.4 SD) % respectively, Followed by those of age group 30-39 years and age group 40-49 years. While the lowest mean of total sperm count, progressive motility, non-progressive motility, total motility count and normal sperm morphology were recorded among those of age group ≥ 50 years, they were as follows; 129 (± 42.5 SD) million, 17.7 (± 8.1 SD) %, and 10.8 (± 4 SD) %, 38.8 (± 21 SD) million and 6.8 (± 2.1 SD) % respectively. The highest mean of immotility 71.5 (± 9 SD) % was recorded among those of age group ≥ 50 years and the lowest mean of immotility 49 (± 9.3 SD) % was recorded among those of age group 20-29 years. All these differences were statistically significant $P < 0.05$ (table 2).

This study clarified a significant decrease in almost all of the parameters among men above 40 versus ≤ 40 years, except sperm immotility which increase.

There were significant negative weak correlations between age and semen volume, sperm concentration, however, good significant negative correlation with total sperm count, progressive motility, non-progressive motility, total motility count and sperm morphology, while positive correlation between age and immotility (table 4).

Table (1): Mean (SD) of age and indices of semen quality among studied group

| Items | Studied group | Males no. 452 |
|--|---------------|------------------|
| Age (years) | | |
| Range | | 20-60 |
| (Mean \pm SD) | | 36 \pm 10.1 |
| Age groups in years | | No. % |
| 20-29 | | 150 (33.2) |
| 30-39 | | 174 (38.5) |
| 40-49 | | 76 (16.8) |
| ≥ 50 | | 52 (11.5) |
| Semen volume (mL) | | |
| Range | | 0.9-4.2 |
| (Mean \pm SD) | | 2.7 \pm 0.9 |
| Sperm concentration | | |
| Range | | 52-187 |
| (Mean \pm SD) | | 89 \pm 20 |
| Total sperm count ($\times 10^6$) | | |
| Range | | 67.14 - 882.96 |
| (Mean \pm SD) | | 243.6 \pm 110 |
| Progressive motility (%) | | |
| Range | | 5-41 |
| (Mean \pm SD) | | 28.7 \pm 8.1 |
| Non Progressive motility (%) | | |
| Range | | 3-30 |
| (Mean \pm SD) | | 15.8 \pm 4.7 |
| Immotility (%) | | |
| Range | | 29-92 |
| (Mean \pm SD) | | 55.45 \pm 11.2 |
| Total motility count ($\times 10^6$) | | |
| Range | | 5.8-494.5 |
| (Mean \pm SD) | | 114.2 \pm 65.7 |
| Morphology (%) | | |
| Range | | 4-39 |
| (Mean \pm SD) | | 25 \pm 9 |

Table (2): Distribution of semen quality among different age groups

| Indices of semen quality | Age groups | 20-29, no.=150 (33.3%) | 30-39, no.= 174 (38.5%) | 40-49, no.= 76 (16.8%) | ≥ 50 , no.= 52 (11.5%) | ANOVA test |
|--|------------|------------------------|-------------------------|------------------------|-----------------------------|------------------|
| | | (Mean \pm SD) | (Mean \pm SD) | (Mean \pm SD) | (Mean \pm SD) | |
| Semen volume (mL) | | 3.01 \pm 0.8 | 2.9 \pm 0.88 | 2.3 \pm 0.76 | 1.6 \pm 0.9 | F=49, p.=0.000* |
| Sperm concentration ($\times 10^6$ / ml) | | 92 \pm 24 | 90.5 \pm 19.2 | 86 \pm 14 | 79.8 \pm 13 | F=5.8, p.=0.001* |
| Total sperm count ($\times 10^6$ / ejaculate) | | 281.6 \pm 114.5 | 265.5 \pm 104 | 197 \pm 72.2 | 129 \pm 42.5 | F=40, p.=0.000* |

| | | | | | |
|--|----------|----------|-----------|----------|------------------|
| Progressive motility (%) | 33.3±6.8 | 30.6±3.8 | 23±7.5 | 17.7±8.1 | F=119, p.=0.000* |
| Non Progressive motility (%) | 17.9±4.5 | 17.3±2.9 | 11.7±3.7 | 10.8±4 | F=85, p.=0.000* |
| Immotility (%) | 49±9.3 | 52±4.4 | 65.4±8.8 | 71.5±9 | F=165, p.=0.000* |
| Total motility count (×10 ⁶ /ejaculate) | 146±67.6 | 128±55 | 71.2±35.5 | 38.8±21 | F=70, p.=0.000* |
| Morphology (%) | 32.7±3.4 | 26.5±4.3 | 18.7±6.2 | 6.8±2.1 | F=45, p.=0.000* |

*P<0.05

Table (3): Distribution of semen quality among volunteers ≤40 and >40 years

| Indices of semen quality | Age groups | | T. test |
|---|-------------------------------|---------------------------------|----------------|
| | no=329 (72.8%) (Mean ± SD) | no.= 123 (27.2%) (Mean ± SD) | |
| Semen volume (mL) | 3±0.9 | 2±0.7 | t=11 p.=0.000* |
| Sperm concentration(×10 ⁶ / ml) | 91±21.5 | 83±13.7 | t=4 p.=0.001* |
| Total sperm count(×10 ⁶ / ejaculate) | 273.2±109 | 164.2±64 | t=17 p.=0.000* |
| Progressive motility (%) | 31.8±5.5 | 20.4±8 | t=15 p.=0.000* |
| Non Progressive motility (%) | 17.5±7.3 | 11.3±3.9 | t=20 p.=0.000* |
| Immotility (%) | 50.7±7.3 | 68.3±9.5 | t=10 p.=0.000* |
| Total motility count (×10 ⁶ / ejaculate) | 136±61 | 55.5±32 | t=14 p.=0.000* |
| Morphology (%) | 29.2±5.1 | 13.7±7.7 | t=24 p.=0.000* |

Table (4): Correlation between age and Semen quality

| Items | Semen volume | Sperm concentration | Total sperm count | Progressive motility | Non Progressive motility | Immotility | Total sperm motility | Morphology |
|-------|--------------|---------------------|-------------------|----------------------|--------------------------|------------|----------------------|------------|
| R | -0.480 | -0.107 | -0.408 | -0.638 | -0.555 | 0.698 | -0.520 | -0.870 |
| p. | 0.000* | 0.023* | 0.008* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* |

4. Discussion

Although significant focus has been placed on female reproductive aging, there is also an age-related decline in sperm function and male fertility. Although “andropause” is not a clearly defined event for men as menopause is for women, there is a decline in testicular function, which includes declining testosterone levels each year (McLachlan, 2000). Semen quality analysis is the preliminary step to know the fertility status of males for assisted reproduction (WHO, 2010).

The present study gives an idea about the changing semen quality between the different decades. This study revealed that aging is characterized by a statistically significant decrease in semen volume, sperm concentration, but increase in sperm immotility at different age groups. Interestingly, semen quality in participants above 40 years tended to decrease statistically with respect to men aged ≤ 40 years. Also, there were significant negative correlations between age and semen volume, sperm concentration.

These results are in accordance with published reports by (Kidd et al., 2001) who evaluated the effects of age on semen parameters and they concluded that advancing age leads to a decline in

semen volume. Also, (Jung et al, 2002) compared semen parameters retrospectively in the elderly (>50 years of age) and in young men (21-25 years of age) and demonstrated a statistically significant decrease in semen volume in elderly males.

Moreover, (Eskenazi et al., 2003) by cross-sectional study sought to characterize the association between age and semen quality. A convenience sample of 97 non-smoking men (aged 22±80 years) without known fertility problems was recruited from a national government laboratory demonstrated that men in their 20s had a median semen volume of 3.0 ml, and there was a significant trend toward lower volumes across age decades with decrease in semen volume by approximately 0.03 mL/year. Also, they found a significant trend toward increased numbers of men with abnormal concentration across age decades.

In addition, (Levitas et al., 2007); in a large study group (a total of 6022 patients), examined the differences in semen parameters between various age groups. They reported that the semen volume gradually and significantly decreased in relation to age. Similarly, (Molina et al., 2010); In 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). They were detecting a significant decrease in seminal volume in relation to age.

(**Eskenazi et al., 2003**) stated that decreased semen volume with age may be caused by seminal vesicle insufficiency, since seminal vesicle fluid contributes most of the ejaculate volume, Changes in the prostate that occur with ageing, such as smooth muscle atrophy and a decrease in protein and water content, may contribute to decreased semen volume and sperm motility. While, (**Molina et al., 2010**) stated 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.

On the contrary, (**Zhu QX et al., 2011**); clarified by a cross-sectional population-based study investigated the relationship between age and semen parameters in Chinese men their study included 998 patients between the ages of 20 and 60. They did not find a declining trend between age and semen volume or sperm concentration. Moreover, (**Kidd et al., 2001**); concluded that advancing age does not affect sperm concentration. Also, (**Jung et al., 2002**); demonstrated a sperm concentrations was not affected by age.

Concerning the total sperm count per ejaculate, which is a variable dependent on the semen volume and sperm concentration per milliliter, the present study found that it gradually decrease with age from values of $281.6 \pm 114.5 \times 10^6$ /ejaculate in age group 20-29 years to values of $265.5 \pm 104 \times 10^6$ /ejaculate, $197 \pm 72.2 \times 10^6$ /ejaculate and $129 \pm 42.5 \times 10^6$ /ejaculate in age groups 30 - 39, 40 - 49 and ≥ 50 years, respectively with statistical significant difference. There was significant negative correlation between age and total sperm count ($r = -0.408$, $p = 0.008$).

These results are in agreement to (**Eskenazi et al., 2003**); who demonstrated that total sperm count decreased significantly across age decades. Also, (**Molina et al., 2010**); detected a statistically significant decrease in total sperm count with age.

On the contrary, (**Zhu QX et al., 2011**); did not find a declining trend between age and total sperm numbers.

In the present study, Peak progressive sperm motility of 33.3 ± 6.8 % and non progressive sperm motility of 17.9 ± 4.5 % were observed in age group 20 - 29 years and gradual reduction was observed in older age groups. In addition, higher sperm motility was detected among men ≤ 40 years compared to those above 40. ($P < 0.05$). Also, there were significant negative correlation between age and progressive sperm motility ($r = -0.638$, $p = 0.000$). and non-progressive sperm motility ($r = -0.555$, $p = 0.000$).

These results are in accordance with (**Kidd et al.,**

2001); who concluded that advancing age leads to a decline in sperm motility. Also, (**Jung et al., 2002**) demonstrated a statistically significant decrease in sperm motility in elderly males.

Furthermore, (**Eskenazi et al., 2003**); demonstrated that semen specimens provided by men in their 20s had medians of 50.0% motility, 29.0% progressive motility and 96.6×10^6 total progressively motile sperm. There were significant trends towards reduced sperm motility across age decades for all three parameters.

In addition, (**Sloter et al., 2006**); performed linear regression analyses of 14 aspects of semen quality measured by computer-assisted semen analysis (CASA) in a non-clinical cohort of 90 non-smoking men, aged 22-80 years, who had no history of infertility or reproductive problems. They found that age-associated declines in CASA-determined motility (0.4% per year, $P \leq 0.001$) with no evidence for age thresholds and no significant association with abstinence duration.

Also, (**Levitas et al., 2007**); found that a statistically significant decrease in sperm motility with aging. Semen parameters were affected most dramatically in individuals over the age of 55, who exhibited significantly lower sperm motility than patients in other age groups. Sperm motility was found to be inversely related to age with peak motility of $44.39 \pm 20.69\%$ at age < 25 years and lowest motility of $24.76 \pm 18.27\%$ at age ≥ 55 years ($P < 0.05$).

Moreover, (**Sunanda et al., 2014**); in a retrospective study a total 730 semen samples were analyzed. Subjects were grouped according to the age (20-29, 30-34, 35-39 and 40-50). This study showed negative correlation of progressive motility ($r = -0.131$, $p < 0.01$) with age. They added that as normal morphology of sperms is essential to gain motility during epididymal transit, it might be difficult to attain higher grade of motility, imperative for conception in older age males. There were reports of increased percentage of abnormal spermatozoa in older age groups than younger groups. Further, deterioration of healthy germ cells in advanced age might be one of the reasons for loss of grade of motility.

Concerning to total motile sperm count, it was found that total motility count decreased from peak mean value of $146 \pm 67.6 \times 10^6$ /ml at age group 20-29 years to mean values of $128 \pm 55 \times 10^6$ /ml, $71.2 \pm 35.5 \times 10^6$ /ml and $38.8 \pm 21 \times 10^6$ /ml at age groups of 30-39, 40-49 and ≥ 50 respectively. With statistical significant difference. There was significant inverse correlation between age and total motile sperm count ($r = -0.52$, $p = 0.000$).

These results are similar to some extent with the results of (**Levitas et al., 2007**); who revealed a

gradual and a significant reduction in total motile sperm with age, the lowest total motile sperm count of $46.68 \pm 53.73 \times 10^6$ was noted at age >55 years.

As regard normal sperm morphology, Peak normal sperm morphology of 32.7 ± 3.4 % was observed in the 20 – 29 years group. A gradual reduction was observed in the following age groups and the lowest value 6.8 ± 2.1 % was recorded among group ≥ 50 years ($P < 0.05$). There was significant negative correlation between age and normal sperm morphology ($r = -0.870$, $p = 0.000$).

These results are in agreement to the results of (Jung et al., 2002) who demonstrated a statistically significant decrease in normal sperm morphology in elderly males. Also, (Levitas et al., 2007); found that the peak percentage of normal sperm morphology was observed among the younger patients. In particular, the highest value was noted among >25 to <30 years old patients with a peak normal morphology of 8.41 ± 6.10 % and lower values were recorded at ages >55 years with 7.30 ± 7.42 % .

Similarly, (Molina et al., 2010) detected that the percentage of morphologically normal spermatozoa evaluated according to WHO criteria decreased significantly in men aged more than 50 years. In addition, (Sunanda et al., 2014); observed negative correlation of normal morphology ($r = -0.324$, $p < 0.01$) with age.

(Molina et al., 2010); stated that the simultaneous diminution in sperm density and the percentages of morphologically normal spermatozoa with aging strongly suggests the occurrence of some level of impairment in the spermatogenic process.

5. Conclusion & Recommendations:

As semen quality is a tool for predicting fertility. These data clearly illustrated an effect on semen parameters among men as they get older.

It important to raise awareness among both clinicians and couples to the risks associated with delayed fatherhood, which may reduce their chance of get children, therefore compromise their quality of life.

Paternal age should be taken in consideration when searching for infertile couples.

Further studies and investigations are needed to demonstrate the mechanisms involved in these changes with age.

Aging effect suggested to be taken into consideration when proposing normal standard values for semen characteristics during routine semen analysis to standardize the population.

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