# In men seeking fertility assistance, defects in sperm capacitation/fertilizing ability are common in all age groups, in contrast, semen volume and motility declined with age

Fady Sharara[1], Cristina Cardona[2], G. Charles Ostermeier[2], Alexander J. Travis[2,3]. [1]Virginia Center for Reproductive Medicine, [2]Androvia LifeSciences, Mountainside, NJ; [3]Cornell University, Ithaca, NY.

## Abstract

**Introduction:** Sperm must capacitate to become fertilization competent. Cap-Score™, which quantifies capacitation status to functionally assess male fertility, prospectively predicts pregnancy. Semen analysis (SA) does not diagnose sperm function defects and fails to predict fertility. Multiple societal factors including education, career, life goals, financial considerations, and health issues are causing couples to delay having children. Delaying parenthood raises several concerns related to reproductive success. It is generally accepted that maternal age is inversely related with fertility and pregnancy outcome. However, the influence of paternal age on male fertility parameters is largely unknown.
**Objective**: The objective of this study was to determine how capacitation ability, as measured by Cap-Score, and traditional semen analysis (SA) measures (Volume, Concentration, Motility) change with paternal age.
**Materials and Methods:** Cap-Score and SA measures were collected from men questioning their fertility (MQF; n=2,652; multicentric design, 35 reproductive endocrinologist (RE) prescribers, n=16 clinics). A Mann-Whitney test was used to compare Cap-Scores between MQF and a population of men with known recent paternity (n=76). MQF were separated into the following age groups 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, & 50+ (n=22, 280, 926, 843, 374, 143, and 64 per group respectively). Mixed model ANOVAs were performed to evaluate associations between SA, Cap-Score, and age groups, and to account for any potential impact of Cap-Score collection kit type within the age groups (n=763 collected at home and n=1,889 collected at the clinic).
**Results:** Men questioning their fertility had reduced capacitation ability (29.2±0.15 vs 35.3±0.88) p<0.001). There was no change in Cap-Score (p=0.916) or concentration (p=0.926) in association with the age groups. In contrast, both semen volume (p=0.008) and the percent of sperm motility (p<0.001) declined with age.
**Conclusions:** Capacitation ability is reduced in MQF when compared to men with known paternity. In MQF and actively pursuing fertility assistance with an RE, motility and volume declined with age. Reductions in capacitation, or sperm fertilizing ability, were equally prevalent across the age groups in MQF. These data show that capacitation ability is sensitive to male fertility issues across age groups and shouldn't be reserved for older men.

Cardona, et al. 2017 Mol Repro and Devel. 84(5),
Moody, et al. 2017. Mol Repro and Devel. 84 (5), 408-422.
Schinfeld, et al. 2018. Mol Repro and Devel. 85 (8-9), 654-664
Sharara, et al. 2020. RBMO. 41(1), 69-79

## Introduction

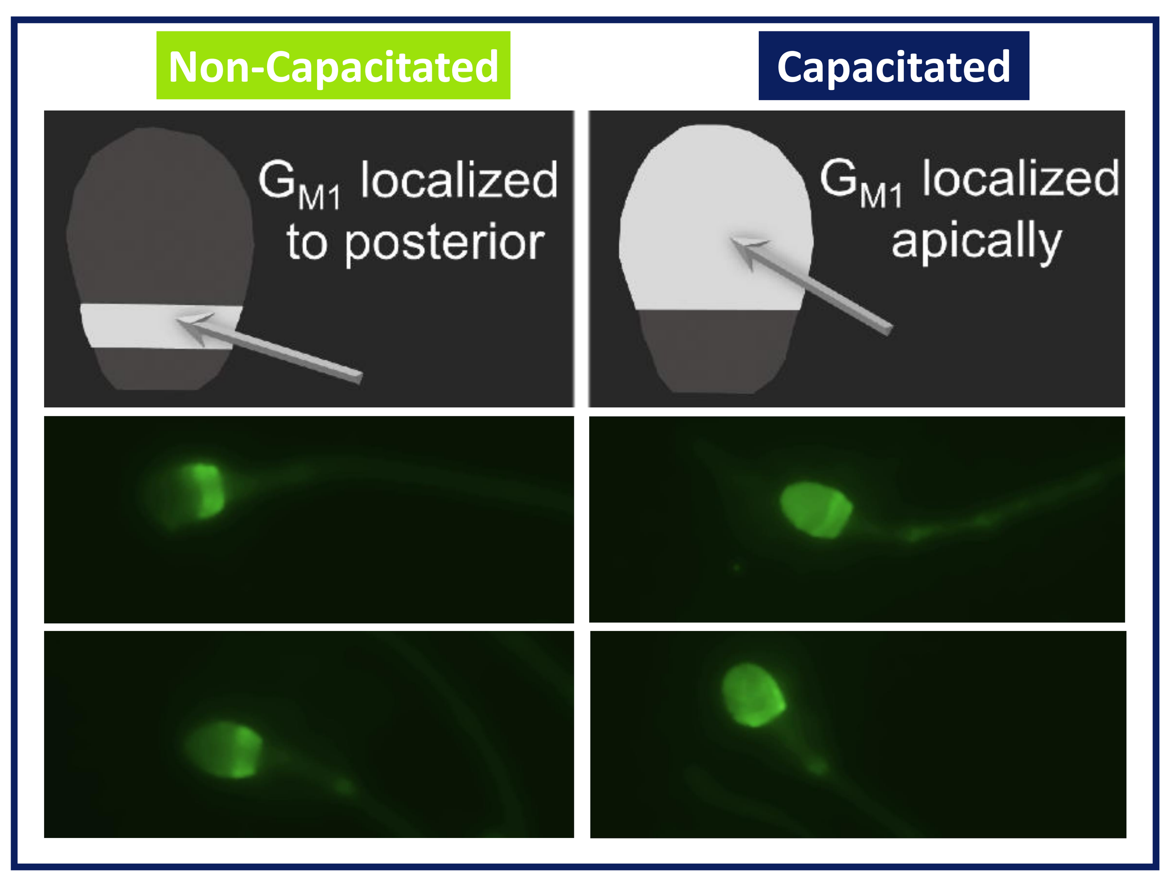


**Figure 1.** ***Cap-Score™.*** Cap-Score™ is defined as the percentage of capacitated sperm within an ejaculate and is determined by distribution patterns of the ganglioside $G_{M1}$ (Moody et al 2017). The images in the left column show respectively, a diagram and fluorescent microscopy images typical of sperm that have not been exposed to, or have not responded to, stimuli for capacitation. The right column shows $G_{M1}$ distributions that are typical for cells that have responded to stimuli for capacitation.

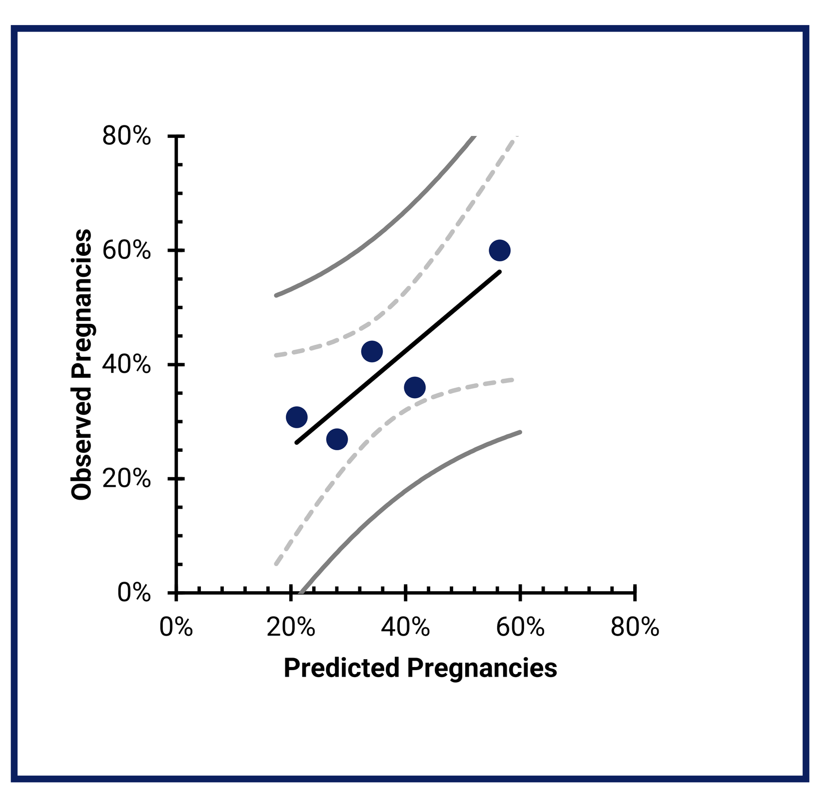


**Figure 2.** ***Cap-Score prospectively predicts pregnancy.*** The relationship between Cap-Score and a man's probability of generating pregnancy (PGP) was defined previously (Schinfeld, et al. 2018). Another prospective study by Sharara et al (2020) tested this relationship. Cap-Score and its associated PGP were determined for 128 new individuals. The couples were then followed over 3 rounds of IUI. The population was ordered by PGP and then divided into 5 equally sized groups (blue dots). No difference was detected between the predicted and observed pregnancies, substantiating the relationship between capacitation ability and a man's fertility. The black line shows the regression equation (RE). The dotted and solid gray lines show the 95% CI for the RE and observed data (OD), respectively.

## Experimental design

Cap-Score and SA measures were collected from men questioning their fertility (MQF; n=2,652; multicentric design, 35 reproductive endocrinologist (RE) prescribers, n=16 clinics). A Mann-Whitney test was used to compare Cap-Scores between MQF and a population of men with known recent paternity (n=76). MQF were separated into the following age groups 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, & 50+ (n=22, 280, 926, 843, 374, 143, and 64 per group respectively). Mixed model ANOVAs and multiple comparisons (Fisher's least significant difference (LSD)) were performed to evaluate associations between SA, Cap-Score, and age groups, and to account for any potential impact of Cap-Score collection kit type within the age groups (n=763 collected at home and n=1,889 collected at the clinic).

## Results

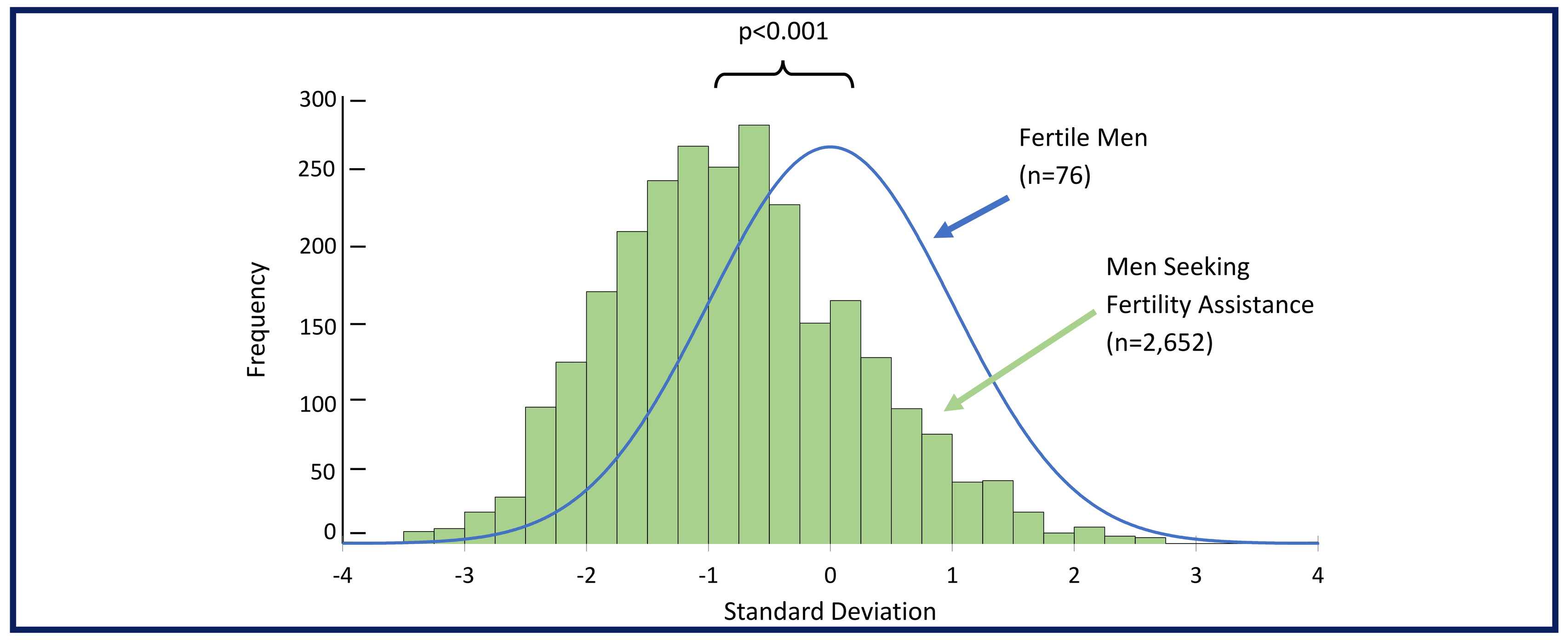


**Figure 3.** ***Comparison of Cap-Scores between men of know fertility and men questioning their fertility (MQF).*** Cap-Scores were collected from MQF at reproductive endocrinology offices (n=2,652; green histogram) and a population of known fertile men (n=76; blue bell curve). Both distributions were standardized so that the Cap-Score mean (35.3) and standard deviation (7.7) of the fertile population was set to zero and one unit respectively. There was a significant reduction in Cap-Scores in MQF (0.0±0.11vs -0.79±0.02; p<0.001).

Type III Sum of Squares analysis (Cap-Score):

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Kit type | 1.0 | 30.699 | 30.699 | 0.496 | 0.481 |
| Age group | 6.0 | 126.435 | 21.073 | 0.341 | 0.916 |
| Kit type*Age group | 6.0 | 392.854 | 65.476 | 1.058 | 0.386 |

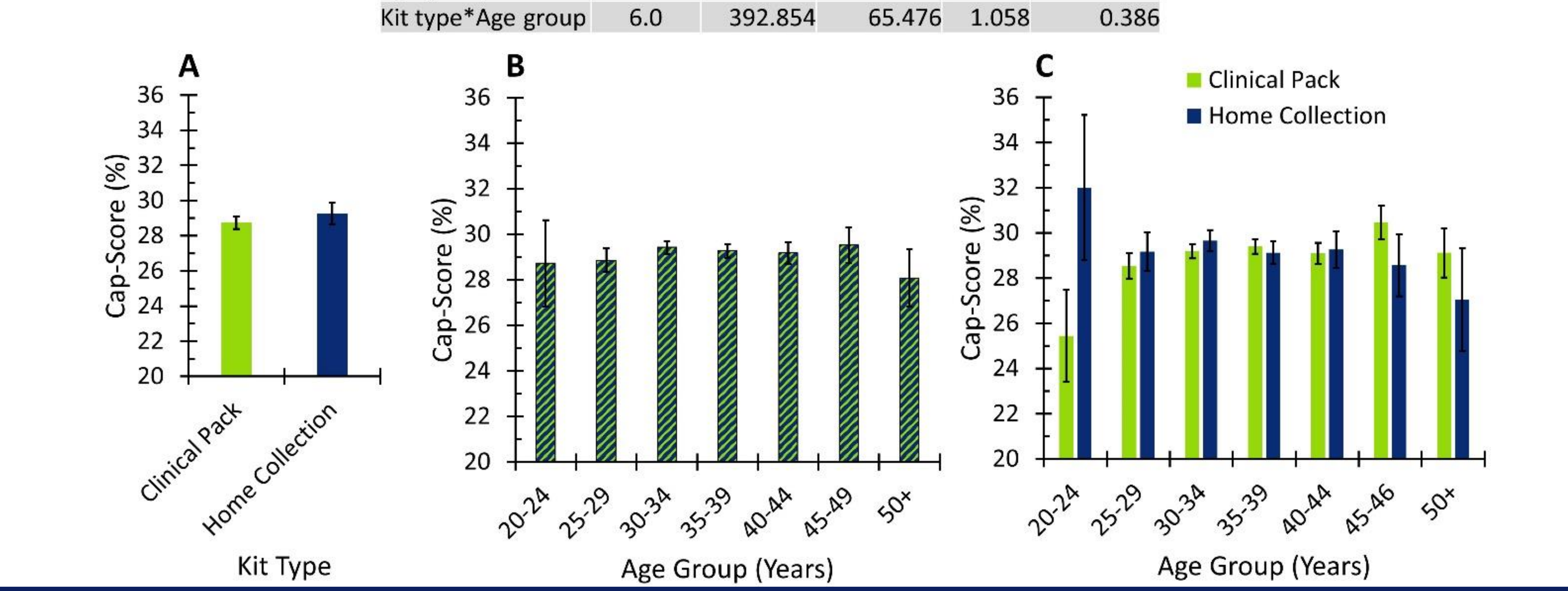


**Figure 4.** ***The relationship between age and Cap-Score in MQF.*** The table shows the type III sum of square analysis for the ANOVA. The bar charts show the Cap-Score least square means (LS) and their standard errors. **Panel A** illustrates that kit type (p=0.481) did not have a significant impact on Cap-Score. No association was detected between the age groups and Cap-Score (p=0.916; **Panel B**). The data shown in **Panel C** demonstrate that there was no significant effect of Kit type within Age Group (p=0.386). Source – independent variable; DF – degrees of freedom; F – the value obtained when running an ANOVA and informs if a *group* of variables are jointly significant; Pr>F – the probability of getting the calculated F statistic and is obtained from an F distribution with degrees of freedom calculated from the number of groups and the total number of subjects in the experiment.

Type III Sum of Squares analysis (Log Concentration):

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Kit type | 1.0 | 0.474 | 0.474 | 3.867 | 0.049 |
| Age group | 6.0 | 0.237 | 0.039 | 0.322 | 0.926 |
| Kit type*Age group | 6.0 | 0.218 | 0.036 | 0.296 | 0.939 |

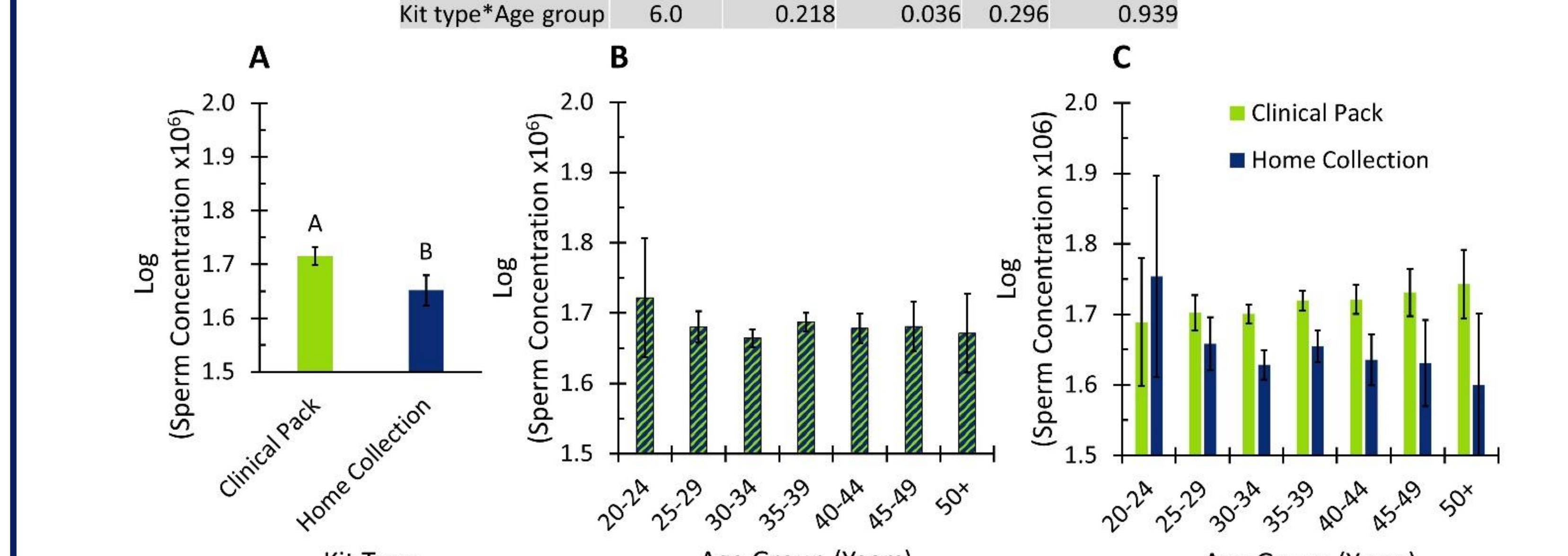


**Figure 5.** ***The relationship between age and concentration in men MQF.*** To normalize the measures of sperm concentration, they were log transformed. The table shows the type III sum of square analysis for the ANOVA. The bar chart shows the Log(Concentration) least square (LS) means and standard errors. A significant relationship was found between log concentration and kit type (p=0.049; **Panel A**). This result was predictable, because home collection enables the assay to be performed on samples having lower concentrations and absolute numbers of sperm. No significant differences were detected in Log(Concentration) among the age groups (p=0.926; **Panel B**) or kit type within age groups (p=0.939; **Panel C**).

Type III Sum of Squares analysis (Motility (%)):

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Kit type | 1.0 | 49021.407 | 49021.407 | 119.372 | 0.000 |
| Age group | 6.0 | 10810.067 | 1801.678 | 4.387 | 0.000 |
| Kit type*Age group | 6.0 | 1600.095 | 266.682 | 0.649 | 0.691 |

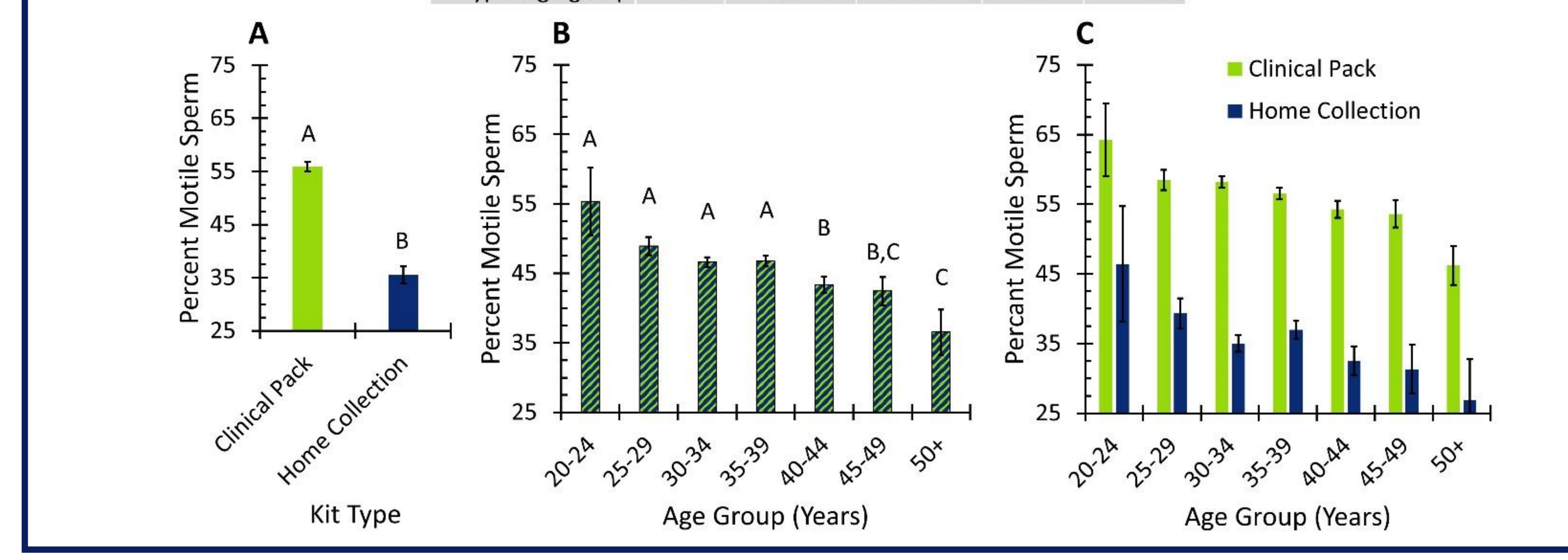


**Figure 6.** . ***The relationship between age and motility in MQF.*** The table shows the type III sum of square analysis for the ANOVA. The bar charts show the percent motile sperm least square (LS) means and standard errors. Significant relationships were detected between percent motile sperm and kit type (p<0.001; **Panel A**) and among the age groups (p<0.001; **Panel B**). In contrast, no relationship was found between percent sperm motility and kit type within age group (p=0.691; **Panel C**). Those groups with different superscripts were deemed unique by Fisher's least significant difference (LSD).

Type III Sum of Squares analysis (Volume):

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Kit type | 1.0 | 0.825 | 0.825 | 0.388 | 0.533 |
| Age group | 6.0 | 37.455 | 6.242 | 2.934 | 0.007 |
| Kit type*Age group | 6.0 | 11.239 | 1.873 | 0.880 | 0.508 |

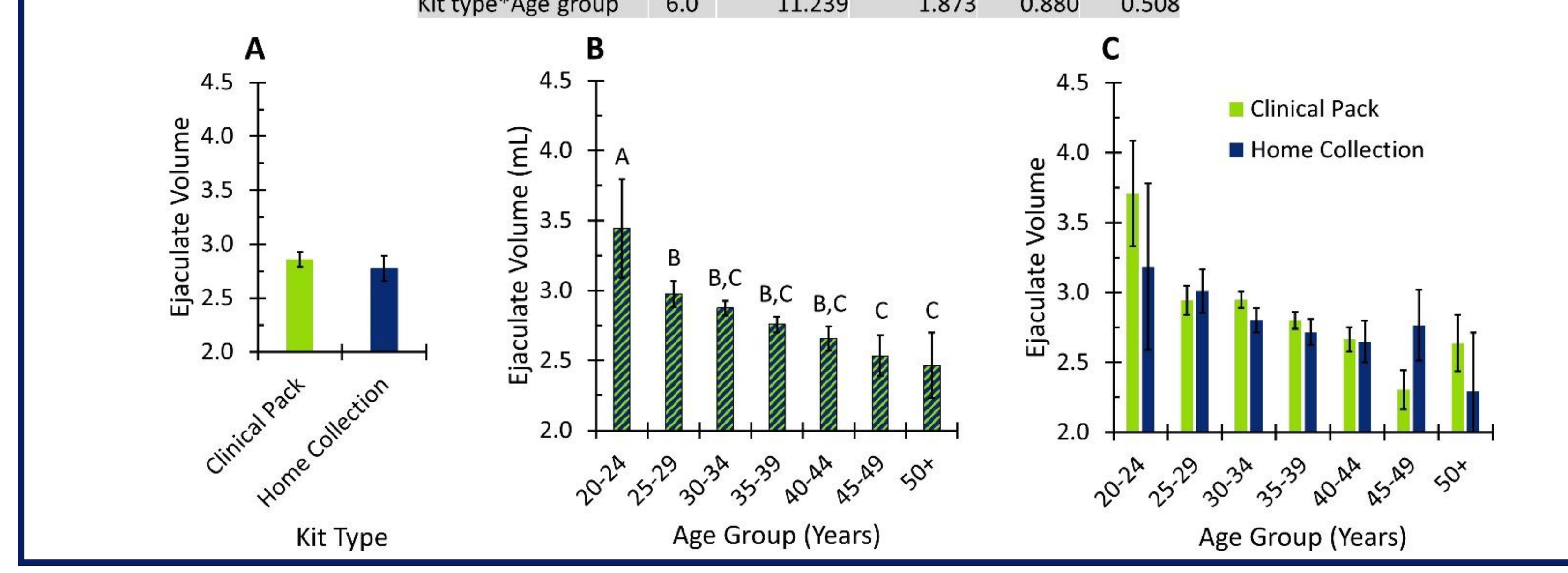


**Figure 7.** ***The relationship between age and ejaculate volume in MQF.*** The table shows the type III sum of square analysis for the ANOVA. The bar charts show the ejaculate volume least square (LS) means and standard errors, as these are most appropriate in multi-way designs and are likely closer to reality than arithmetic means. There was no significant difference in ejaculate volume between kit type (p=0.533; **Panel A**) and among the kit type within age group (p=0.508; **Panel C**). In contrast, ejaculate volume declined with age (p=0.007; **Panel B**). Those groups with different superscripts were deemed unique by Fisher's least significant difference (LSD).

## Conclusions

- Sperm fertilizing ability is reduced in MQF when compared to men with known paternity.
- In MQF who are actively pursuing fertility assistance, motility and volume declined with age.
- Reductions in capacitation, or sperm fertilizing ability, were equally prevalent across the age groups in MQF. These data show that capacitation ability is sensitive to male fertility issues across age groups and shouldn't be reserved for older men.

Cap-Score results provided by Androvia LifeSciences