

Does Erotica by Virtual Reality Improve Sperm Quality?

Study Protocol and Statistical Analysis Plan

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Sperm samples were donated in private rooms dedicated for the purpose and equipped with a touch screen showing erotic material. The men were asked to donate as usual and self-report the amount of time (in hours) since their last ejaculation (the abstinence period). Every other donation, donors rolled dice to determine whether a VR headset would be used; with the subsequent donation performed with the opposite condition. To maximize privacy and minimize stress on the donors, the amount of time the donors took to produce a sperm sample (donation period) was recorded as the number of seconds from closing the door to the private room to when it was opened again.

Each semen sample was weighed to determine ejaculate volume and allowed to liquefy at room temperature for up to 1 hour. After liquefaction, aliquots were loaded in duplicate onto Makler counting chambers (Sefi-Medical, Israel) and observed at 200x magnification using an Olympus CX41 microscope (Olympus, Japan). Measurements of sperm concentration, motility and motile sperm concentration were made using a MICROPTIC, S.L. (Barcelona, Spain) Computer Assisted Sperm Analysis system with at least 500 cells counted per analysis. No analysis of sperm morphology was performed.

Statistical Analyses

Data was anonymized prior to analyses and no data was excluded (Supplementary Material Table S1). We used linear mixed models (LMMs) to assess the relationship between VR-use and ejaculate volume, donation period and total motile sperm count (TMSC) for each ejaculate sample, while controlling for several factors that might also influence ejaculates: (i) abstinence period, (ii) donor age, (iii) donor BMI, (iv) day of the year (season), and (v) location (donation site). In each model, we included anonymized donor identity as a random effect to avoid pseudo-replication as each donor provided multiple samples. The interaction between VR-use and abstinence period was initially included in models as abstinence period is known to increase ejaculate size and quality [16,17] though sometimes a decrease has been observed. We considered it to be at least plausible that the influence of VR might diminish as abstinence period increased and both ejaculate size and quality approached their maxima for each donor. We removed those interaction terms from models when p-values for the interactions was >0.20 .

To investigate the relative strength of plausible causal relationships among the variables in our LMMs, we used piecewise structural equation modeling. To construct this model, we assumed that VR use might have a direct effect on ejaculate size, donation period and TMSC, and that ejaculate volume and donation period might have direct effects on

We used R version 4.2.0 for analyses, with packages *lmer* and *lmerTest* for LMMs. For interaction plots we used the *sjPlot* package. For piecewise structural equation modeling, we used the *pSEM* package with the *lme* function (*nlme* package) for mixed models and anonymized donor identity nested within location as random effects. To predict the magnitudes of effects from the full LMMs, we used the *ggpredict* function in the *ggeffects* package. All such effects are reported as mean [95%CL] calculated by setting the other predictors in the full models at their mean values.