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Adjusting for abstinence time in semen analyses: some considerations

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Background

There is a need to study factors that influence male fertility, as fertility is associated not only with reproductive success, but also long-term health (Tvrda *et al.*, 2015). Semen quality, often characterized by parameters such as sperm concentration or motility, is usually used to evaluate male fertility, despite said parameters not always correlating with pregnancy rates or infertility (Bonde *et al.*, 1998; Guzick *et al.*, 2001). In order to accurately estimate associations between exposures and semen quality, one often needs to control or adjust for confounding factors that may influence the relationship of interest. Sexual abstinence time is one factor for which many researchers adjust.

Abstinence time is informative for understanding male reproductive endocrine functioning and diagnosing conditions such as a congenital absence of the vas, a hypoandrogenic state, retrograde ejaculation, or ejaculatory duct obstruction (Turek, 2014). Abstinence time is further of concern in semen analysis because it may influence parameters such as semen volume, sperm concentration, and possibly, DNA fragmentation (De Jonge *et al.*, 2004). Though easy to assess through patient self-report, it is an arguably a crude measure because its relationship with semen parameters will vary considerably depending on recent ejaculatory frequency as well as its unit of measurement (e.g., days, hours).

The World Health Organization (WHO) recommends collection of semen samples after two to seven days of sexual abstinence due to the within- and between-subject variability in semen quality (World Health Organization, 2010). Some studies not only limit abstinence time before collection, but also adjust for it in regression models. Limiting a study analysis

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Authors' contribution

KAM, EY, and SLM developed the manuscript concept. YL performed the literature search. KAM, KK, and ENC performed the literature review. KAM analyzed the review results. KAM, TCP, and SLM drafted the manuscript. All authors revised the manuscript for important intellectual content and clarity. All authors approved the final manuscript for submission.

to only men who were abstinent for a specified period is one possible form of adjustment, through the selection of study participants. For example, in requiring all participants to be sexually abstinent for 2 to 7 days before semen collection, a researcher is selecting participants from only one stratum of abstinence time and disregarding the rest (e.g., less than 2 days, one year). Depending on one's research question, abstinence time may or may not act as a confounder and one must consider the implications of selecting on or statistically adjusting for it. Adjustment for factors that do not act as confounders can bias associations of interest and may or may not improve their precision (Schisterman *et al.*, 2009). For more on confounding and selection, we recommend Greenland *et al.* (2008) and Rothman *et al.* (2008).

Avoiding practices which may negatively affect causal interpretation or precision will better allow us to identify exposures associated with male fertility. Our goal was to 1) explore how abstinence time is handled across studies in leading journals, 2) discuss examples in which one should avoid adjustment, and 3) identify potential solutions if adjustment is unavoidable.

How do studies handle abstinence time?

We conducted a literature review in PubMed for articles that mention the terms “abstinence” or “semen” and were published between October 2014 and September 2015 within eight leading epidemiologic and reproductive health journals (*Andrology*, *Fertility and Sterility*, *Human Reproduction*, *Reproductive Sciences*, *Epidemiology*, *American Journal of Epidemiology*, *Paediatric and Perinatal Epidemiology*, *International Journal of Epidemiology*). Our initial search returned 131 articles; after review, we excluded 44 irrelevant articles. Of the 87 included articles, 82% described using WHO guidelines for their semen analyses, but some were unclear about using the guidelines specifically for abstinence time restriction prior to collection. Forty-five percent of the articles limited abstinence time before semen collection, but did not statistically adjust for it; 18% statistically adjusted for abstinence time with or without limiting it before collection; and 37% did not comment on abstinence time restriction or adjustment. Among the studies not commenting on abstinence time, the majority reported using WHO guidelines and therefore, adjustment for abstinence time via study design before semen collection may be inferred. Bjorndahl and colleagues similarly noted that many studies on semen quality fail to report complete information on sample collection; they recommend authors and editors ensure that abstinence time is adequately described, though they also recommend all samples be collected following 2 to 7 days of abstinence and do not comment on the collection of information such as recent ejaculatory frequency (Bjorndahl *et al.*, 2016).

These data suggest that adjustment for abstinence time, either by selection or statistical adjustment, is common. Most studies did not discuss their reasoning for limiting abstinence time or adjusting for it, but issues of variability in semen parameters and convention in literature were mentioned by some researchers.

To adjust or not adjust for abstinence time?

Depending on the research question of interest, it may or may not be appropriate to select on or adjust for abstinence time. Here we discuss several such scenarios. Specifically, we consider adjustment for 1) a factor that is associated with the outcome of interest, but not the exposure and 2) a factor that may lie on a causal pathway between an exposure and an outcome (i.e., caused by the exposure and in turn, cause the outcome, also known as mediation).

Scenario 1

Abstinence time is often adjusted for when it predicts variability in a semen parameter outcome, but is not associated with the exposure of interest (Figure 1, Panel A). For example, in evaluating the role of dietary fiber on sperm concentration, one may want to adjust for abstinence time because it is associated with the outcome, even if it likely does not also affect fiber intake (i.e., is a confounder). Adjusting for a factor that is only associated with an outcome may improve precision in linear models, but can reduce precision in a logistic model (Robinson and Jewell, 1991). Furthermore, effect estimates from noncollapsible models (e.g., logistic) may change after adjustment due solely to a noncollapsibility effect (Pang *et al.*, 2013). Measures of association, such as risk ratios, are said to be *collapsible* if the unconfounded overall estimate of interest in one's population is equal to a weighted average of the effect estimates calculated within the strata of another variable for which you are adjusting (e.g., within levels of abstinence time or body mass index); in the case of the odds ratio, these overall and weighted estimates may not be equivalent due to a noncollapsibility property rather than the presence of confounding (see Hernán *et al.*, 2011 and Greenland *et al.*, 1999b). Therefore, it is important to consider the relationship that abstinence time has with one's exposure and outcome as well as the statistical approach and type of model to be used when identifying the appropriate set of variables for adjustment.

Scenario 2

Abstinence time may also act as a mediator on a causal pathway between an exposure and a semen quality outcome (Figure 1, Panel B). In these situations, it is especially important to define the research question of interest and carefully consider the implications of adjustment for a mediating factor, as it may change the causal question being answered. An exposure such as metabolic syndrome may causally influence erectile dysfunction (McVary, 2007), which could affect abstinence time and subsequently, sperm concentration. Metabolic syndrome may also influence concentration through other causal pathways that do not include erectile dysfunction or abstinence time. If the total effect of metabolic syndrome on sperm concentration is of interest, it cannot be estimated when a potential causal mechanism (e.g., mediation by abstinence time) is being adjusted for—via selection, stratification, or statistical adjustment.

Furthermore, adjustment in this case would not produce an effect estimate for metabolic syndrome on sperm concentration that is “independent” of abstinence time—i.e., the direct effect of metabolic syndrome that works through causal pathways other than erectile

dysfunction—unless strong assumptions about confounding are met. If there is unmeasured confounding, adjustment for a mediator may actually bias this direct effect one is trying to estimate (for more on mediation and these assumptions, see Richiardi *et al.*, 2013; VanderWeele, 2009; and VanderWeele, 2016).

However, we recognize that adjustment for abstinence time via selection is often “forced,” in that researchers may use semen samples from a clinic that follows WHO semen collection procedures. This restriction allows for easier comparison to semen parameter standards. Nonetheless, selecting on a mediator will still complicate or bias the estimation of total and direct effects. By oversampling on participants who were abstinent for two to seven days, one can unintentionally alter the primary exposure distribution (exposure enrichment) because a causal relationship between the exposure and abstinence time exists (e.g., men with more severe metabolic syndrome may be more likely to be excluded if they were abstinent for more than seven days due to erectile dysfunction; Ahrens *et al.*, 2015).

Potential solutions

Carefully defining the causal question of interest is imperative in determining the appropriate adjustment strategy. Estimation of the direct effect may be the relevant question of interest and if so, suitable methods should be used for estimation (e.g., mediation analyses). Marginal structural models and sensitivity analyses for unmeasured confounding (e.g., bias factors) can be used to estimate direct effects, even when some confounding assumptions are violated (VanderWeele, 2009; VanderWeele, 2016). When abstinence time acts as a mediator and selection on it is “forced,” inverse probability weights may be used in an attempt to correct the bias in the total effect due to oversampling on the mediator (Ahrens *et al.*, 2015). Sensitivity analyses should be performed to estimate the range of possible total effects given assumptions about one’s sampling strategy and uncertainty with measurement. Causal diagrams are also a great tool to visualize the relationships between abstinence time, an exposure, and an outcome (Greenland *et al.*, 1999a). These diagrams, in combination with available statistical methods, can help maximize precision and minimize bias when evaluating semen quality.

In conclusion, we urge researchers using semen analysis data to carefully consider study and analytic designs with regard to abstinence time. By using appropriate adjustment, we can better identify factors that influence male fertility.

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Figure 1.
Causal diagrams reflecting scenarios in which adjustment for abstinence time must be done cautiously