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Published in:
Advances in Physiology Education

DOI:
[10.1152/advan.00131.2019](https://doi.org/10.1152/advan.00131.2019)

Publication date:
2020

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):
Land, S. C., & Booth, D. (2020). Systematic review and meta-analysis as a structured platform for teaching principles of experimentation. *Advances in Physiology Education*, 44(3), 276-285.
<https://doi.org/10.1152/advan.00131.2019>

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Systematic Review and Meta-analysis as a Structured Platform for Teaching Principles of Experimentation.

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29 *Objectives & Overview*

30 Access to knowledge has never been easier in the internet age and so it is important that students
31 develop skills to discriminate undependable information from reliably investigated research. We have
32 created an exercise which teaches good research practice by exploring the history, ethics and design of
33 clinical trials. Students apply their understanding of these principles through an assessed systematic
34 review and meta-analysis (SRMA) exercise. Here, a clinically themed hypothesis is tested using a
35 structured literature search in conjunction with an eligibility matrix to map study design, ethics, subject
36 selection, randomization & blinding, methodological standards, study power and other potential sources
37 of inter-study heterogeneity. Data extracted from selected studies is used to produce a forest plot with
38 an aggregated effect size, confidence range and measure of inter-study heterogeneity. A funnel plot is
39 then used in conjunction with the eligibility matrix to evaluate study bias tendency and, in this way,
40 students reflect upon the factors which promote disparate conclusion-making among studies with a
41 common research focus. This exercise produced a normally distributed grade-profile across three
42 academic year cohorts and comparison of individual exercise grade with year-long aggregated average
43 suggested students who performed less well on conventional assignments engaged successfully with the
44 systematic nature of this assessment. Those opting to use this format for their final year capstone
45 project also performed above their grade point average from the preceding year. We suggest that SRMA
46 offers a readily applied method for students to quantitatively explore how differences in experimental
47 research practices influence study dependability.

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51

52 *Introduction*

53 Scientific objectivity and critical thinking skills are often taught through small-group reflective activities
54 such as journal clubs, critical writing workshops, mock grant committee-style peer review panels or
55 seminar reviews (e.g. 5). These tend to focus on high-impact articles in a research field, published over a
56 defined time-line, with compare-and-contrast discussion around assumptions, experimental design,
57 analysis, conclusions and next steps. Pedagogic evaluation of these methods suggests that students
58 make measurable gains in cognitive and critical thinking skills and that teaching methods tend to
59 diversify to promote student engagement with the activity (25). These approaches may, however, tend
60 to foster the impression that science advances purely by conclusive experimentation or fortunate
61 discovery and that studies reporting neutral or negative results are less valuable or, in some way,
62 flawed. This is a concern because publication bias in favour of positive research outcomes is believed to
63 be fuelling a data reproducibility crisis in the life sciences and commonly employed literature-based
64 teaching techniques may not give adequate attention to this issue (13-15).

65 Systematic review compliments these approaches by encouraging students to view research as a
66 continuum, where positive, neutral and negative results of differing magnitudes are reported from
67 different research locations over time. Rather than selecting studies based on concluding results or
68 impact, a structured literature search is used to identify all primary research articles reporting data
69 around a selected hypothesis, regardless of individual study outcome. These are screened for strengths
70 of study design before outcomes are assessed using meta-analysis to obtain a measure of effect size
71 based on the weighted contribution of each study (21). By aggregating effect sizes across several related
72 studies, statistical power increases to the point where large or small biological effects can be
73 discriminated, with factors that drive differences in reported outcomes (inter-study heterogeneity)
74 evaluated retrospectively. In this way, emphasis is placed on the experimental principles which
75 underpin each study rather than bottom-line results. Here, we describe an adaptation of this systematic

76 review and meta-analysis (SRMA) approach as an exercise for undergraduate biomedical students which
77 encompasses teaching of research ethics, clinical trial regulation and bias management strategies along-
78 side a structured approach to hypothesis testing using meta-analysis.

79

80 *Learning Objectives*

81 The objective is to review the modern history of human experimentation which has driven the
82 development of ethical frameworks for clinical testing and the design of clinical trials. This establishes
83 the background knowledge necessary to conduct an independent systematic review and meta-analysis
84 exercise.

85 *Specific Learning Outcomes*

86 After completing this activity, the student should be able to:

- 87 • Describe the key historical events and developments in ethical reasoning which underpin
88 present day regulation of human and animal experimentation.
- 89 • Use advanced search engine strategies to identify primary research literature which may be
90 used to test a specific hypothesis
- 91 • Use the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow
92 sheet to report the triage of studies for inclusion in a meta-analysis.
- 93 • Understand the forms of bias which can influence data collection and analysis and develop an
94 eligibility matrix to screen articles for adherence to good research practices.
- 95 • Extract data from selected research articles which may be used to generate a forest plot using
96 categorical or continuous data.
- 97 • Describe and interpret data patterns revealed by a forest plot.

- 98 • Apply this information to a funnel plot and use the output to interpret sources of variation
99 which occurs between studies.
- 100 • Explore the effect of removing studies which contain identified sources of bias upon data
101 distribution and intra-study heterogeneity on the forest plot
- 102 • Explain the physiological mechanisms which underpin the effects revealed by the forest plot.

103

104 *Activity Level*

105 We use this activity as a guided learning exercise for students in the third and fourth year of
106 undergraduate study in the biomedical sciences (Scottish Credit Qualification Framework (SCQF) Levels 9
107 (equivalent to BSc Ordinary Degree) and 10 (equivalent to BSc Honours Degree), however, the clinical
108 emphasis of the activity also carries relevance for medical students. This exercise provides appropriate
109 training for quantitative literature-based research as part of an independent capstone project in the
110 final year of undergraduate study paving the way for advanced postgraduate study. The exercise runs
111 with a class size of around 100 students.

112 *Prerequisite Student Knowledge*

113 Students should have practical experience of experimental design gained from laboratory practical
114 sessions as well as a fundamental grasp of physiology and pharmacology. We use R as a platform for
115 teaching statistical analysis and so a basic understanding of R commands and grounding in statistical
116 principles is an advantage.

117 *Time and Resources Required*

118 The exercise runs over 3 workshops each of 2hrs duration. The first session covers the history and ethics
119 of clinical trials, the second explains the principles and approach to meta-analysis and the third is

120 computer-based session covering the process of meta-analysis in R (Fig 1.). Students spend a total of
121 45hrs in face-to-face teaching and completing their final SRMA report.

122

123 METHODS

124 *Instructions*

125 *Workshop 1: History and Ethics of Clinical Trials.* The first workshop provokes discussion about
126 the purpose, history and ethics of clinical trials by explaining the timeline of events that led up to
127 present-day regulation of human and animal experimentation. This begins with an introduction to
128 James Lind’s “Treatise on the Treatment of Scurvy”, published in 1753 (19), which is the earliest
129 documented use of systematic literature review in conjunction with a clinical trial (his successful, but
130 misinterpreted attempt to identify a cure for scurvy (1)). This leads to a discussion of Bradford-Hill’s
131 streptomycin and tuberculosis study as the first case controlled randomised clinical trial design (20) and
132 the relevance of Bradford Hill’s Disease Causation Criteria to modern epidemiology (11). Development
133 of the ethical framework governing clinical trials is presented through the events of World War II which
134 led to the 1948 Nuremberg Code, Thalidomide testing and the 1962 Kefauver Amendments, the 1964
135 Declaration of Helsinki followed by the Tuskegee Syphilis Experiment and the principle of informed
136 consent laid out in the 1979 Belmont Report. We explore what happens when clinical trials go wrong
137 using examples from the University of Pennsylvania Ornithine Transcarbamylase (OTC) adenovirus gene
138 therapy trial, the TGN1412 humanised monoclonal antibody trial as well as contemporary events
139 reported in the media. The workshop ends with a discussion of the 3R Principle of Replacement,
140 Reduction and Refinement as it relates to the use of animals in scientific procedures.

141 *Workshop 2: How to Conduct a Systematic Review and Meta-analysis.* This workshop establishes the
142 principle that systematic review coupled with meta-analysis provides an overall estimate of effect size

143 and variance that is based upon the weighted outcomes of multiple studies testing a similar hypothesis.

144 Students are taken stepwise through the meta-analysis process:

- 145 1. Establishing a single hypothesis.
- 146 2. Screening the literature for appropriate studies. Emphasis is placed on study design which must
147 include steps which have been taken to minimize experimenter bias (eg randomization of
148 treatments to subjects, concealment, blinding of treatments, full data collection).
- 149 3. The use of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)
150 together with the PRISMA Screening Checklist.
- 151 4. Construction of a matrix to report inclusion criteria and reporting quality.
- 152 5. How to extract data from studies and dealing with categorical and continuous data
- 153 6. Forest plot interpretation
- 154 7. Funnel plot measurement of heterogeneity

155 *Workshop 3: IT session with practise data sets.* The purpose of the IT session is to familiarise students
156 with the process of creating and interpreting forest and funnel plots before testing a hypothesis of their
157 own. A basic level of competence with R is assumed but since commands, with explanatory notes, are
158 provided, it is possible for those with no experience to complete the analysis. Part 1 of the workshop
159 focuses on meta-analysis with count data using a systematic review examining if BCG vaccination
160 reduces risk of tuberculosis (TB) in children (24). Part 2 analyses continuous data exploring the
161 effectiveness of reducing unnecessary antibiotic use for hospital inpatients (9). By the end of this
162 session, students have all the necessary information to test a novel hypothesis of their own. Teaching
163 support information for this session is provided in Supplementary
164 Material.S1.(<https://doi.org/10.6084/m9.figshare.11674110>).

165

166 *Assessment:* Learning outcomes were assessed through a SRMA exercise which tested the following
167 hypothesis:

168 H₁: Sperm concentration is lower in smokers compared to non-smokers in human males of reproductive
169 age.

170 This topic was selected because, i) the subject focus is concise but is reported over an extended time-
171 line and from different geographical locations, ii) standardised measurements of semen quality (eg
172 concentration, volume, motility) are widely reported and simple for students to identify in the literature,
173 iii) the number of articles students would be expected to screen is not excessive and, iv) the subject
174 matter promotes understanding of a wide range of biological processes.

175 Students reported their results using a proforma which dispersed marks across 6 steps of the SRMA
176 process (Supplementary Table.S2;<https://doi.org/10.6084/m9.figshare.11674113>). An example
177 response to each step, together with a commentary, is provided in RESULTS. A grading rubric which
178 explains each category and unit of assessment was made available to students in advance of the
179 exercise and served to guide markers through the assessment (Supplementary Table.S3
180 <https://doi.org/10.6084/m9.figshare.11674122>).

181

182 RESULTS

183 Example responses to the 6 components of the assessed exercise are provided as follows:

184 **1. How did you search and screen for selected research articles? (15% of marks).**

185 Students provide a breakdown of their search strategy using the PRISMA Flow Diagram. An advanced
186 search in Pubmed using ((smoke[Title] OR smoking[Title])) AND (sperm[Title] OR semen[Title]) yields 140
187 articles; with an additional 13 articles from other sources, the total number of articles for the initial

188 screen was 153 which proved to be a manageable volume for our students. The screen yields several
189 recent meta-analyses on the topic of smoking, tobacco and fertility (eg 3) which should be noted for
190 comparison in later analysis but not included in the primary literature search. Search engine filters
191 should be used to eliminate irrelevant articles and, for practical purposes, those which are not free-to-
192 view (instructor should emphasize that this is not normal SRMA practise). An example PRISMA triage is
193 shown in Fig. 2; the aim is to identify a short-list of publications that contain suitable data for a forest
194 plot. The primary review follows a standard SRMA reporting process with the exception that no article is
195 eliminated during eligibility screening process since this will be used by the student to evaluate sources
196 of inter-study heterogeneity later in the exercise. 29 articles were identified through this process and
197 are referenced in Supplementary Material.S4 (<https://doi.org/10.6084/m9.figshare.11698473>) together
198 with data to be used in the meta-analysis. Each study is ordered by year of publication and identified by
199 a letter of the alphabet for ease of interpretation in figures.

200

201 **2. Assess your Articles for Eligibility and Create a Matrix (10 % of marks).**

202 The instructor-led workshops include discussion of the bias containment strategies which are key to
203 clinical trial design and owe their origins in Bradford Hill's original randomised case-controlled study of
204 streptomycin and tuberculosis (20). Students demonstrate their understanding of this by creating an
205 eligibility matrix which lists features of experimental design which they identify as important for each
206 article in the meta-analysis (Fig 3). Their matrix is used in later stages of the assignment to identify
207 causes of inter-study heterogeneity that affect their forest and funnel plot analysis.

208

209 **3. Create and interpret a Forest Plot (25% of marks).**

210 The forest plot was developed by Lewis and Ellis, 1982 (17, see 16 for a historical perspective) to
211 determine an estimated effect size based on the proportionate contribution of studies testing the same
212 hypothesis and has since been adopted as the standard method of evaluating effects across multiple
213 studies, conducted at different times and in different geographical locations. It facilitates interpretation
214 of a pooled point estimate and overall variation at a glance and, with assessment of intra-study
215 heterogeneity (10,18), provides a powerful, structured approach for evaluating multi-study tests of a
216 common hypothesis.

217 *Example analysis:* Students are instructed to extract mean, standard deviation and n for smokers and
218 non-smoker sperm concentration from their selected articles. The continuous data protocol explored in
219 Workshop 3 is used to generate a forest plot using data from studies identified in their literature search.
220 An example forest plot is shown in fig 4, where mean sperm concentration (10^6 cells.ml⁻¹), standard
221 deviation (SD) and subject numbers (Total columns) are shown for the 29 studies investigating smoking
222 effects on sperm concentration listed in Supplementary Material.S4. Mean differences (MD) in sperm
223 concentration between smokers and non-smokers are given on the right of the graph together with 95%
224 confidence intervals and study weighting for fixed and random effects models. A summary aggregate
225 analysis is given in the bottom two lines of the plot. Smoking and non-smoking subjects total 6159 and
226 11517 respectively. The fixed effect model assumes near identical study designs across all articles such
227 that inter-study differences arise from chance sampling variation alone. It reports that that smoking
228 reduces sperm concentration by 3.7×10^6 cells.ml⁻¹ and that there is 95% confidence that the true value
229 will lie within the range of $(-4.9$ to $-2.6) \times 10^6$ cells.ml⁻¹. The random effects model assumes that
230 differences in the approach used by each study (eg cross sectional, prospective, retrospective
231 experimental design and sampling differences) will introduce additional causes of variance above the
232 natural pattern assumed by the fixed effects model (23). This model reports that smoking reduces
233 sperm concentration by 4.45×10^6 cells.ml⁻¹ with 95% confidence limits from $(-8.7$ to $-0.2) \times 10^6$ cells.ml⁻¹.

234

235 An analysis of Inter-study heterogeneity is provided in the lower left of the plot. I^2 reports the
236 proportion (%) of the X^2 statistic which is not explained by the variation within the studies
237 (<50=moderate-to-low heterogeneity; >51= high heterogeneity). τ^2 reports the variance of the true
238 effect sizes based on the random effects model and the probability value reports likelihood of variation
239 between studies. In this example, $p < 0.01$ indicates high probability that each study reports an outcome
240 which differs from the mean result so the fixed and random effect models should be treated with
241 caution. Further investigation of inter-study heterogeneity should be performed using Funnel Plot
242 analysis.

243 **4. Create and Interpret a Funnel Plot (25% of marks).**

244 Funnel plots provide a visual evaluation of the precision of studies in the forest plot. By plotting the
245 standard error against mean difference, a distribution is obtained where high-powered studies cluster
246 either side of the mean result near the plot apex and low-powered studies occur towards the base. Since
247 95% confidence limits vary inversely with study precision, a funnel-shaped confidence interval boundary
248 is created which enables studies deviating outside these limits to be identified and investigated (18).

249 *Example Analysis:* The data used to generate the forest plot produces the funnel plot shown in Fig. 5.
250 Most studies cluster close to the apex around the mean difference, however some lie outside the 95%
251 confidence intervals suggesting that the overall data set is heterogeneous and could include extremes of
252 bias. Students are instructed to use their eligibility matrix to evaluate application of bias containment
253 strategies across their selected articles. Fig 3 identifies numerous possible causes of inter-study
254 heterogeneity in this data set, however, recent comment in the field has highlighted poor compliance
255 with internationally agreed semen analysis protocols as a problem (2,4). Students are encouraged to
256 explore this in their own data by testing if inter-study heterogeneity decreases among studies which cite

257 the 5th (2010) edition of the “WHO Laboratory Manual for the Examination and Processing of Human
258 Semen” (26). A refined forest plot constructed from 8 articles which follow these criteria shows that
259 inter-study heterogeneity remains high (I^2 90%, $P < 0.01$) but the funnel plot now reveals three studies
260 which lie beyond the 95% confidence limits (B,C,J in Fig. 6). In common with most of the studies used in
261 this analysis, few report adequate steps to contain bias and, so, given that there are no unique reasons
262 to exclude any one study, the final part of the analysis tested the effect of removing Study B as the most
263 distant outlier to the aggregated mean distance (Fig 7). This had the following effects: 1) mean
264 difference values for the fixed and random effect models now closely agree and increase from those
265 given in Figs. 4 & 5. 2) I^2 is $< 50\%$ and the likelihood of studies deviating from the mean effect value is
266 now 38%. Taking the random effects model as the most conservative estimate, it is now safe to
267 conclude that smoking reduces sperm concentration by 8.4×10^6 cells.ml⁻¹. WHO lower reference limits
268 for normal sperm concentration are 15 (12-16) $\times 10^6$ cells.ml⁻¹ (5th centile, 95% confidence limits) (7) and
269 so it can be concluded that smoking induces a 44% reduction in fertility below this lower reference
270 value.

271 5. Linking the constituents of cigarette smoke to spermatogenesis (25% of marks).

272 Students are asked to summarise the molecular mechanisms which could link smoking behaviour to
273 spermatogenesis. We impose a strict limit of 150 words to encourage a focussed, abstract-style
274 paragraph. Students are required to describe one or more mechanisms which make a clear link between
275 smoking behaviour and the molecular regulation of spermatogenesis with informative graphical
276 abstracts encouraged. For information about the effects of cigarette constituents on spermatogenesis,
277 the reader is referred to refs 6,8 & 22.

278 6. References (5% of marks)

279 References are cited according to instructions from a leading journal in the field. For this exercise, this
280 was the journal, Human Reproduction (Oxford Academic, Oxford, UK)

281 *Evaluation of Student Work*

282 The pro-forma report and marking rubric guide the student through the task, award a weighted grade
283 for different skill components and facilitate feedback. We do not require students to perform an
284 exhaustive literature search but they should aim to demonstrate appropriate use of advanced search
285 engines, triage reporting methodology and develop a suitable eligibility screen. The analysis shown here
286 identified a total of 29 relevant publications from 1992-2016, however, students typically based their
287 reports on approximately half this number, with 5 stipulated as the lowest acceptable number of
288 articles. The literature search process is time-consuming and so students were encouraged to use
289 discussion boards to share search strategies, though exchange of reference material was not permitted.

290 In our hands, this exercise produces a normal grade distribution with a median score of 60-63% from a
291 pooled cohort of 298 students from 3 consecutive years (Fig 8A). The normal distribution suggests
292 effective discrimination of student ability across the range of grades available with no evidence of
293 kurtosis that might arise from variable student engagement or differences in assessment approaches.
294 Individual performance on this exercise was examined by plotting the grade difference for the SRMA
295 assignment (assignment grade point minus year-long running average grade point) against year-long
296 performance for each student. Regression analysis suggests a modest relationship ($r^2=0.31$) whereby
297 individual attainment tended to be greater among students whose overall year-long attainment was
298 otherwise low (Fig 8B). We did not evaluate the reasons behind this but note that active learning
299 exercises, of the type described here, increase performance among several metrics of learning
300 attainment (12). It may be that SRMA encompasses a structured approach to literature review which
301 facilitates engagement across the diverse learning abilities and styles. Finally, we examined retention of

302 SRMA learning outcomes by following the performance of students who opted to complete a SRMA
303 capstone project in a subsequent year of study. Here, individual performance was assessed as the
304 difference between capstone project grade and personal aggregate performance in the previous year of
305 study. Individual performance was found to be consistent between SRMA and other project formats
306 (wet laboratory, science communication or bioinformatics) (Figure 8C) suggesting that students were
307 able to retain their understanding of SRMA from one year to the next and apply this to varied questions
308 in the biosciences to a standard that matched other capstone project formats.

309

310 *Common issues and errors.*

- 311 1. A set of frequently asked questions (FAQ) has been collated from our SRMA on-line discussion
312 board which addresses most issues encountered by students on this exercise (see
313 Supplementary Material.S5 (<https://doi.org/10.6084/m9.figshare.11702067>)).
- 314 2. Data conversions. Studies may report different measures of variance, requiring conversion to
315 standard deviation. Similarly, studies may categorise smoking intensity of subjects in different
316 ways and so, for simplicity, we advise students to determine an aggregated mean and SD of all
317 smoking intensities. Conversion advice is provided in the FAQ's.
- 318 3. Selection of criteria for eligibility matrix. Students are referred to supporting lecture material on
319 this topic and are encouraged to consider the Bradford-Hill selection criteria (11). Some may,
320 however, become aware of the PRISMA checklist which runs to over 27 selection criteria. We
321 advise students to avoid being too prescriptive in their selection of eligibility criteria but that
322 they should focus on use of strategies to contain bias, adherence to measurement standards if
323 relevant to the field, study size and use of techniques to assess study power.

324 4. Interpretation of forest plots. Students tend to gloss over the detail of forest plots to focus on
325 the bottom-line result. This is compounded by confusion between appropriate use of fixed and
326 random effects models and interpretation of heterogeneity information. Most commonly,
327 students mis-interpret significant intra-study heterogeneity P values as an indication of effect
328 size significance. We suggest careful guidance in the IT workshop together with advice offered
329 on the discussion board as the best approach to address these issues.

330 5. Interpretation of Funnel Plots. As with Forest Plots, students tend to focus on the basic
331 interpretation of the plot without attempting a deeper analysis of the data. It is important to
332 emphasize that no paper may be removed from the analysis without justified cause, as reflected
333 in the eligibility matrix. Students should recognise that there are limits to this analysis and that
334 inter study heterogeneity may be an issue which affects the wider field.

335
336

337 *Limitations/Adaptations*

338 The following should be considered when adapting this format to other topics: 1. Subject relevance
339 should complement wider teaching goals. In the example described in this article, smoking and semen
340 quality facilitated discussion of the modern history of epidemiology as well as the toxicology of tobacco
341 smoke constituents. 2. The hypothesis should be precise and encourage focus on a single measured
342 parameter or outcome. 3. The literature base for the topic should be manageable in size, readily
343 identified using advanced search methods and freely accessible. The PRISMA report (Fig 2) indicates the
344 volume of literature analysis and screening expected of students in the present exercise. 4. Consider if
345 data will be extracted from tables or graphs. Is the data continuous or categorical? Our exercise
346 required extraction of continuous data that is commonly reported among standard measurements of
347 semen quality. Categorical data may be converted to an odds ratio or similar as described in the

348 Supplementary Material.S1. 5. Are there known causes of inter-study heterogeneity which may provide
349 the opportunity for critical evaluation of experimental approaches in the field? For our exercise,
350 heterogeneity arose primarily from varied adherence to a standard methodology over time, however,
351 there are some notable geographical differences in the reporting of smoking as a positive or negative
352 influence upon sperm concentration.

353 By running this exercise in the penultimate year of study, our intention was to provide students with the
354 skills to conduct an independently researched SRMA capstone project in the subsequent final year. To
355 date, 19 SRMA project topics in neuroscience, pharmacology and physiology have been completed at
356 our institution suggesting that this format may be readily applied to a range of topics. Capstone project
357 titles which are the basis for the data set in figure 8C are given in Supplementary Material.S6
358 (<https://doi.org/10.6084/m9.figshare.11674107>)

359 *Conclusion*

360 The SRMA exercise described here provides an opportunity for structured, quantitative evaluation of a
361 focussed question using the peer reviewed scientific literature. The process requires students to
362 consider the factors which underpin reliable study design, management of bias and data reporting. Our
363 analysis of student performance reveals that the active learning attributes of the exercise may benefit
364 students who tend to perform less well other forms of assessment. The format provides a suitable
365 grounding for in-depth exploration of diverse topics through a capstone project in the advanced years of
366 the undergraduate curriculum.

367

368 *Additional Resources*

369 Students are referred to Cochrane.org for access to articles explaining the SRMA process and to the
370 Cochrane Library, a searchable database of evidence-based clinical studies. Students and instructors
371 may also find the Collaborative Approach to Meta-Analysis and Review of Animal Data from
372 Experimental Studies (CAMARADES) website helpful which can be accessed at
373 www.dcn.ed.ac.uk/camarades/contact.html. The CAMARADES collaboration provides support for SRMA
374 of data from experimental animal studies.

375 *Acknowledgements*

376 We thank undergraduate Biomedical Science, Neuroscience, Pharmacology and Physiological Sciences
377 students at Dundee University whose participation and feedback on this exercise has informed its
378 development.

379

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435 human semen. 5th ed. Geneva: World Health Organization, 2010.
- 436

437 **Figure Legends**

438 **Figure 1.** Timeline of topics covered in the SRMA exercise.

439

440 **Figure 2.** Preferred Reporting Items for SRMA (PRISMA) flow diagram illustrating initial triage of articles
441 to produce the final selection of articles to be used for the meta-analysis.

442

443 **Figure 3.** Eligibility matrix showing map of experimental design, bias management, reporting of ethics
444 and adherence to methodological standards for 29 studies to be incorporated into the meta-analysis.

445

446 **Figure 4.** Forest plot showing study size, mean, standard deviation (SD), mean difference (MD), 95%
447 confidence intervals (CI) and fixed or random model weighting from 29 studies reporting effects of
448 smoking on sperm concentration. Mean and SD are reported as 10^6 cells.ml⁻¹. Pooled values were used
449 from studies which reported sperm parameters values for mild, moderate and heavy smoking habits.
450 Fixed and random effect summary outcomes are shown.

451

452 **Figure 5.** Funnel plot showing the relationship between individual study standard error and mean
453 difference. Letters denote studies listed in Table 2. Long dash, fixed effect model median; short dash,
454 random effect model median; angled dashed lines, region within which 95% of studies would be
455 expected to lie in absence of intra-study variability and publication bias (calculated as the fixed effect
456 summary log mean difference $\pm 1.96 \times$ standard error of summary log mean difference).

457

458 **Figure 6.** Reduced forest (A) and funnel (B) plots based on 8 studies which cite the 5th edition of the
459 WHO methodology (2010) (26). Labelling details are as indicated in figures 2 & 3.

460

461 **Figure 7.** A minimum forest (A) and funnel (B) model which produces a statistically insignificant level of
462 heterogeneity between studies, achieved by removal of study [B].

463

464 **Figure 8. A.** Frequency histogram showing distribution of grades as percentage categories for an
465 identical SRMA exercise conducted over two consecutive years. N=298. Grade distribution is indistinct
466 from a normal distribution (Kruskal-Wallis Rank Sum test $X^2 = 18$, d.f= 18, p = 0.46) Note that grade bin
467 categories are non-linear at range extremities. **B.** Individual performance on this meta-analysis exercise.
468 Δ grade point average (Δ GPA) was determined as the difference between exercise grade point and year-
469 long aggregated average grade point as determined on a 23-point scale. Regression is linear ($y = -0.57x$
470 $+ 8.7$; $R^2 = 0.31$, $F(1,179)=77.6$, $p<0.001$, $N=181$). Long dash line indicates zero intercept; Data points
471 above this line indicate an exercise performance which is above the individual's running average for the
472 year. **C.** Individual performance of SRMA capstone projects (N=19) compared to all other project formats
473 (N= 205). Δ grade point average (Δ GPA) was determined as the difference between capstone project
474 grade point and year-long aggregated average grade point during the penultimate year as determined
475 on a 23-point scale. Dash line indicates zero intercept; data points above this line indicate exercise
476 performance above the individual's running average for the penultimate year of study. An independent-
477 samples t-test was conducted to compare Δ GPA between those taking the SMRA capstone project
478 versus other project formats. There was not a significant difference in the scores for SMRA capstone
479 project (M=1.11, SD=2.38) and other project (M=1.50, SD=2.50) conditions; $t(222)= -0.68$, $p = 0.49$.

480



- **Clinical Trial Historical References**
King Nebuchadnezzar II and the Royal Diet (605-562 BC)
James Lind Treatise on the Scurvy (1753).
- **Ethics & The Modern History of Clinical Trials**
Nuremberg – Thalidomide & the Kefauver Amendments
Declaration of Helsinki – Tuskegee Syphilis Trial - Belmont report.
- **Bradford-Hill Criteria** – limiting bias in clinical trials.
- **When Clinical Trials go wrong** – Pennsylvania Adenovirus OTC Trial, TGN1412 Northwick Park Hospital.
- **Ethical developments for the next generation**
Open discussion
Animals in experimentation and the 3Rs.

- **An introduction to Meta Analysis**
Purpose and origins.
- **Statistical Power & evaluating effects across studies.**
- **Use of PRISMA & reporting of eligibility criteria when evaluating studies for inclusion in meta analysis.**
- **Extracting the data.**
- **Interpreting Forest plots.**
- **Dealing with intra-study heterogeneity & interpreting funnel plots.**

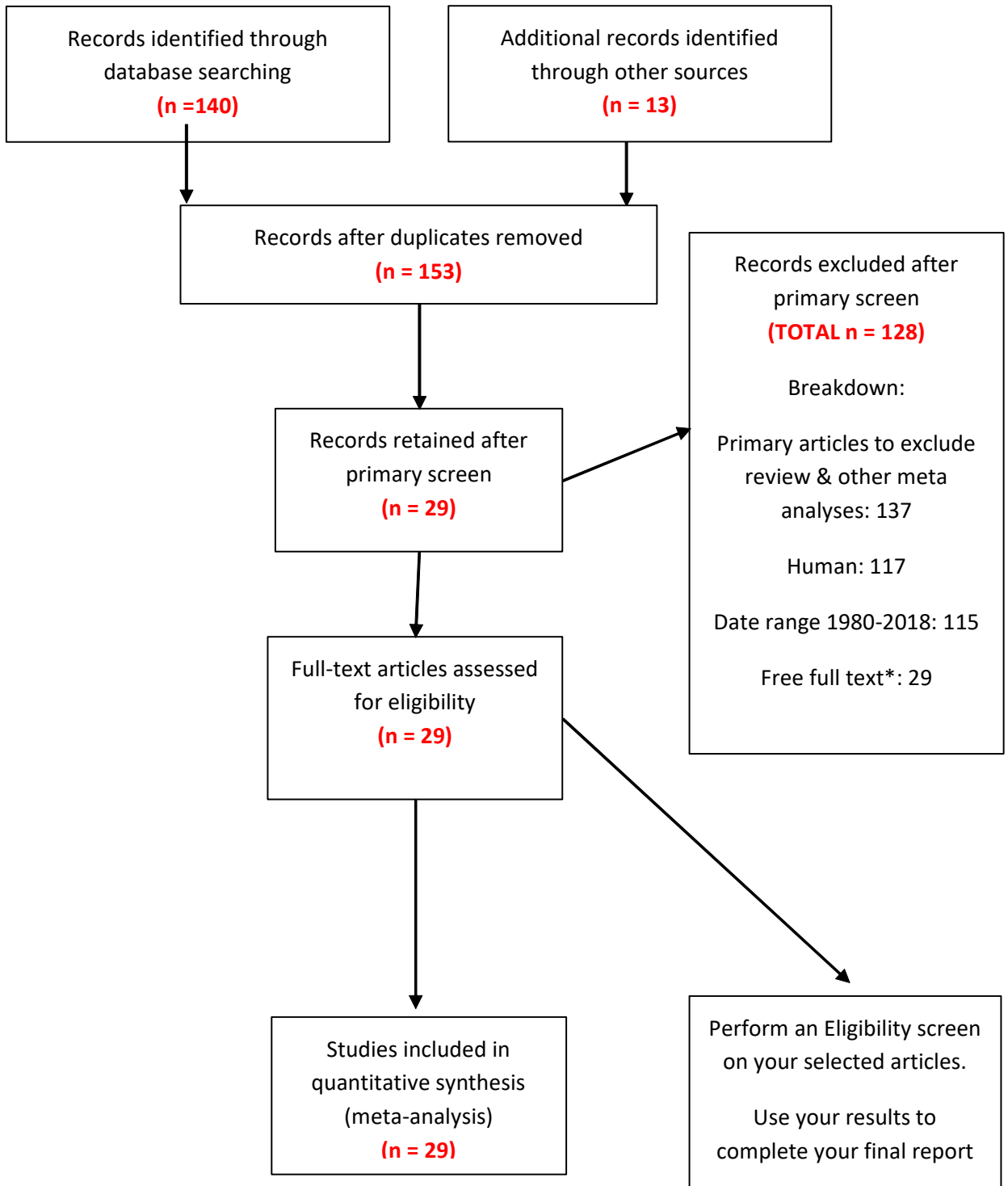
- **Creation of Forest & Funnel plots** using categorical and continuous data.
- **Meta Analysis with Categorical Data:** BCG vaccination reduces risk of tuberculosis (TB) in children. Focus on Roy *et al*, 2014 (24).
- **Meta Analysis with Continuous Data:** Do antibiotic prescribing interventions improve patient recovery times? Focus on Davey *et al*, 2017 (9).
- **Designing your study question.**

- **Independent systematic review & meta analysis** using clinical trial literature.
- **Proforma Style Report (Meta Analysis)** – 4.5 credits (45 hours of effort).
- **CMA - ethical questions arising from clinical trials** (incorporated into wider assessment).



PRISMA Flow Diagram

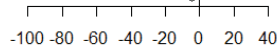
- Identification
- Screening
- Eligibility
- Included



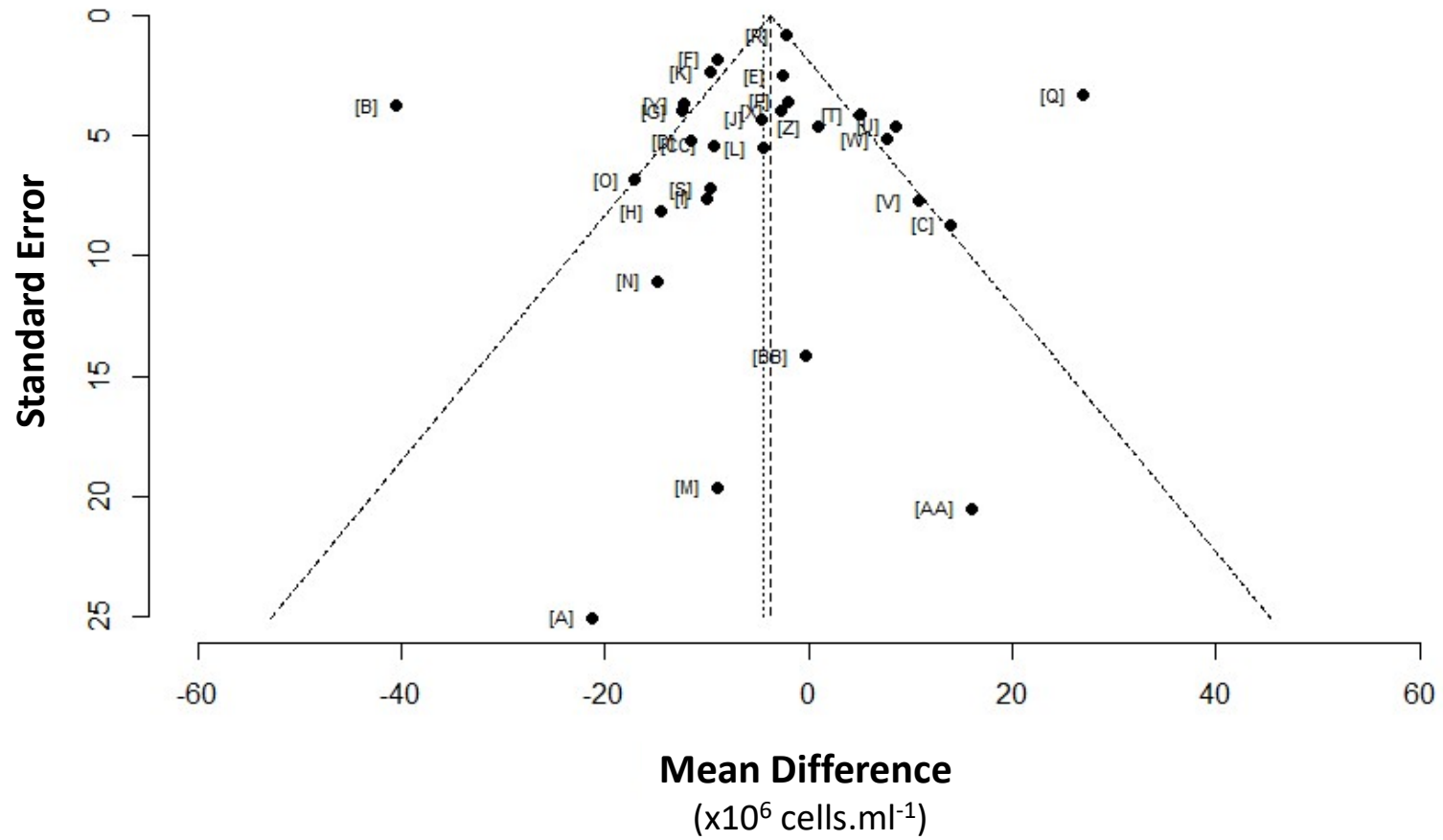
Reference	Cross-sectional Study	Prospective Study	Retrospective Study	Blinded Participant Selection	Randomised Study Design	Blinded Analysis	Inter-observer coefficient of variation reported	Independent Statistical Analysis	Power Analysis Performed	Biological Gradient (Exposure in pack years)	Excludes Subjects with Pathologies related to Infertility	Ethical Statement	Cites Standardised Methodologies before 2010	Cites Standardised Methodology (WHO 5th Edition, 2010)
[A] Antoniassi et al, 2016	✓			x	x	x	x	x	x	x	✓	✓	x	✓
[B] Asare-Anane et al, 2016	✓			x	x	x	x	x	✓	✓	✓	✓	x	✓
[C] Moretti et al, 2014		✓		x	x	x	x	x	✓	✓	✓	✓	x	✓
[D] Yu et al, 2014		✓		?	?	x	x	x	✓	✓	✓	✓	x	✓
[E] Zhang et al, 2013		✓		x	x	x	x	x	✓	✓	✓	✓	x	✓
[F] Meri et al, 2013		✓		x	x	?	x	x	x	✓	✓	x	✓	x
[G] Caserta et al, 2012		✓		x	x	x	x	x	x	✓	✓	✓	x	✓
[H] Davar et al, 2012	✓			x	x	x	x	x	x	✓	x	x	x	✓
[I] Joo et al, 2012	✓			✓	✓	✓	x	✓	x	✓	✓	✓	✓	x
[J] Taha et al, 2012		✓		x	x	x	x	x	x	✓	✓	✓	x	✓
[K] Al-Matubsi et al, 2011	✓			✓	x	x	x	✓	x	x	✓	✓	x	✓
[L] El-Melegy et al, 2011		✓		x	x	x	x	x	x	✓	✓	✓	✓	x
[M] Chohan et al, 2010	✓			x	x	x	x	x	x	x	✓	✓	✓	x
[N] Collodel et al, 2010		✓		x	x	x	x	x	x	✓	✓	✓	✓	x
[O] Liu et al, 2010		✓		x	x	x	x	x	x	✓	✓	✓	✓	x
[P] Kumosani et al, 2008	✓			✓	x	x	x	x	x	x	✓	x	x	✓
[Q] Lopez-Teijon et al, 2007		✓		✓	x	?	✓	x	x	✓	?	✓	✓	x
[R] Ramlau-Hansen et al, 2007		✓		✓	x	x	x	x	x	✓	✓	✓	✓	x
[S] Richthoff et al, 2007	✓			x	x	x	✓	x	x	✓	✓	✓	✓	x
[T] Khademi et al, 2005		✓		x	x	✓	x	x	x	✓	✓	✓	x	x
[U] Ozgur et al, 2005		✓		x	x	x	x	x	x	✓	✓	✓	x	x
[V] Sobreiro et al, 2005		✓		x	x	x	x	x	x	✓	✓	x	✓	x
[W] Pasqualotto et al, 2004		✓		x	x	x	x	x	x	✓	✓	?	?	x
[X] Martini et al, 2004		✓		x	x	x	x	x	x	✓	✓	x	✓	x
[Y] Kunzle et al, 2003		✓		x	x	x	x	x	x	✓	✓	✓	✓	x
[Z] Trummer et al, 2002		✓		x	x	x	x	✓	x	✓	✓	✓	✓	x
[AA] Wallock et al, 2001	✓			x	x	x	x	x	x	✓	✓	✓	✓	x
[BB] Shen et al, 1997		✓		x	x	x	x	x	x	✓	x	✓	✓	x
[CC] Lewin et al, 1991	✓			x	x	x	x	x	x	✓	x	x	✓	x

Study	Experimental			Control			Mean Difference	MD	95%-CI	Weight (fixed)	Weight (random)
	Total	Mean	SD	Total	Mean	SD					
[A] Antoniassi et al, 2016	20	69.90	56.7000	20	91.20	96.7000		-21.30	[-70.43; 27.83]	0.1%	0.7%
[B] Asare-Anane et al, 2016	95	52.60	18.0000	45	93.20	22.1000		-40.60	[-48.00; -33.20]	2.3%	4.2%
[C] Moretti et al, 2014	39	78.00	46.0000	71	64.00	39.0000		14.00	[-3.05; 31.05]	0.4%	2.7%
[D] Yu et al, 2014	147	57.50	40.9000	175	69.00	52.6000		-11.50	[-21.72; -1.28]	1.2%	3.8%
[E] Zhang et al, 2013	737	54.40	50.2000	775	57.00	46.7000		-2.60	[-7.49; 2.29]	5.2%	4.6%
[F] Meri et al, 2013	396	34.70	28.6000	564	43.70	26.5000		-9.00	[-12.57; -5.43]	9.9%	4.7%
[G] Caserta et al, 2012	200	54.50	42.8000	448	66.90	54.0000		-12.40	[-20.16; -4.64]	2.1%	4.2%
[H] Davar et al, 2012	53	86.70	41.1000	98	101.30	58.5000		-14.60	[-30.62; 1.42]	0.5%	2.9%
[I] Joo et al, 2012	49	64.20	44.4000	13	74.20	15.2000		-10.00	[-24.93; 4.93]	0.6%	3.1%
[J] Taha et al, 2012	80	72.00	27.9000	80	76.70	26.6000		-4.70	[-13.15; 3.75]	1.8%	4.1%
[K] Al-Matubsi et al, 2011	111	48.80	15.5000	93	58.50	18.1000		-9.70	[-14.37; -5.03]	5.7%	4.6%
[L] El-Melegy et al, 2011	30	61.70	21.4000	30	66.20	21.4000		-4.50	[-15.33; 6.33]	1.1%	3.7%
[M] Chohan et al, 2010	20	125.00	73.0000	58	134.00	83.0000		-9.00	[-47.47; 29.47]	0.1%	1.0%
[N] Collodel et al, 2010	153	61.30	70.3000	118	76.20	102.9000		-14.90	[-36.55; 6.75]	0.3%	2.2%
[O] Liu et al, 2010	68	58.60	38.7000	79	75.70	44.1000		-17.10	[-30.49; -3.71]	0.7%	3.3%
[P] Kumosani et al, 2008	23	37.30	12.5000	66	39.40	19.9000		-2.10	[-9.11; 4.91]	2.6%	4.3%
[Q] Lopez-Tejzon et al, 2007	241	56.90	43.9000	731	30.00	46.1000		26.90	[20.43; 33.37]	3.0%	4.4%
[R] Ramlau-Hansen et al, 2007	1052	54.10	19.1000	1490	56.30	21.7000		-2.20	[-3.80; -0.60]	49.3%	4.8%
[S] Richthoff et al, 2007	85	65.30	51.7000	217	75.00	67.0000		-9.70	[-23.85; 4.45]	0.6%	3.2%
[T] Khademi et al, 2005	48	35.00	25.3000	122	30.00	21.0000		5.00	[-3.07; 13.07]	1.9%	4.1%
[U] Ozgur et al, 2005	198	63.00	44.2000	98	54.40	33.2000		8.60	[-0.41; 17.61]	1.5%	4.0%
[V] Sobreiro et al, 2005	176	118.70	86.7000	324	107.90	74.6000		10.80	[-4.37; 25.97]	0.5%	3.0%
[W] Pasqualotto et al, 2004	367	116.70	74.9000	522	109.00	76.0000		7.70	[-2.36; 17.76]	1.2%	3.8%
[X] Martini et al, 2004	372	48.10	74.9000	3194	50.90	51.0000		-2.80	[-10.61; 5.01]	2.1%	4.2%
[Y] Kunzle et al, 2003	655	67.70	74.9000	1131	79.90	75.0000		-12.20	[-19.41; -4.99]	2.4%	4.3%
[Z] Trummer et al, 2002	478	58.80	74.9000	517	57.90	70.8000		0.90	[-8.17; 9.97]	1.5%	4.0%
[AA] Wallock et al, 2001	24	89.00	74.9000	24	73.00	67.0000		16.00	[24.21; 56.21]	0.1%	0.9%
[BB] Shen et al, 1997	28	70.20	74.9000	32	70.50	2.4000		-0.30	[-28.06; 27.46]	0.2%	1.6%
[CC] Lewin et al, 1991	214	45.70	74.9000	382	55.00	33.2000		-9.30	[-19.87; 1.27]	1.1%	3.7%
Fixed effect model	6159			11517				-3.73	[-4.85; -2.61]	100.0%	--
Random effects model								-4.45	[-8.72; -0.17]	--	100.0%

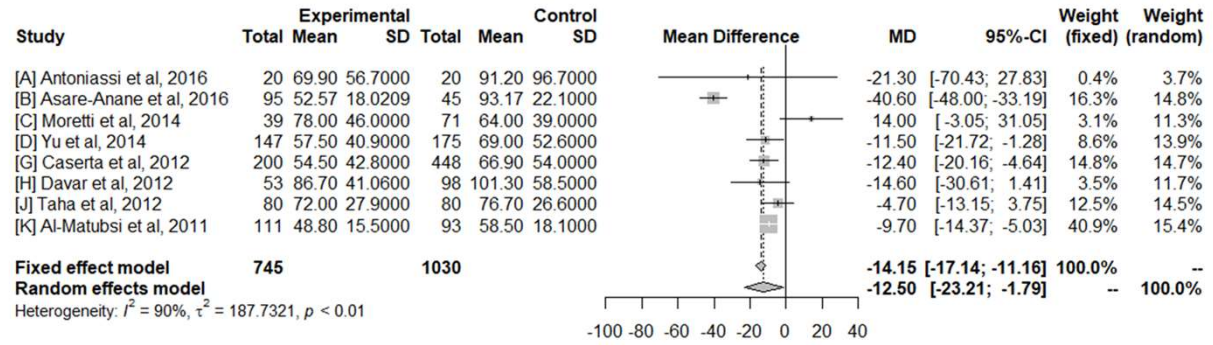
Heterogeneity: $I^2 = 89\%$, $\tau^2 = 97.9307$, $p < 0.01$



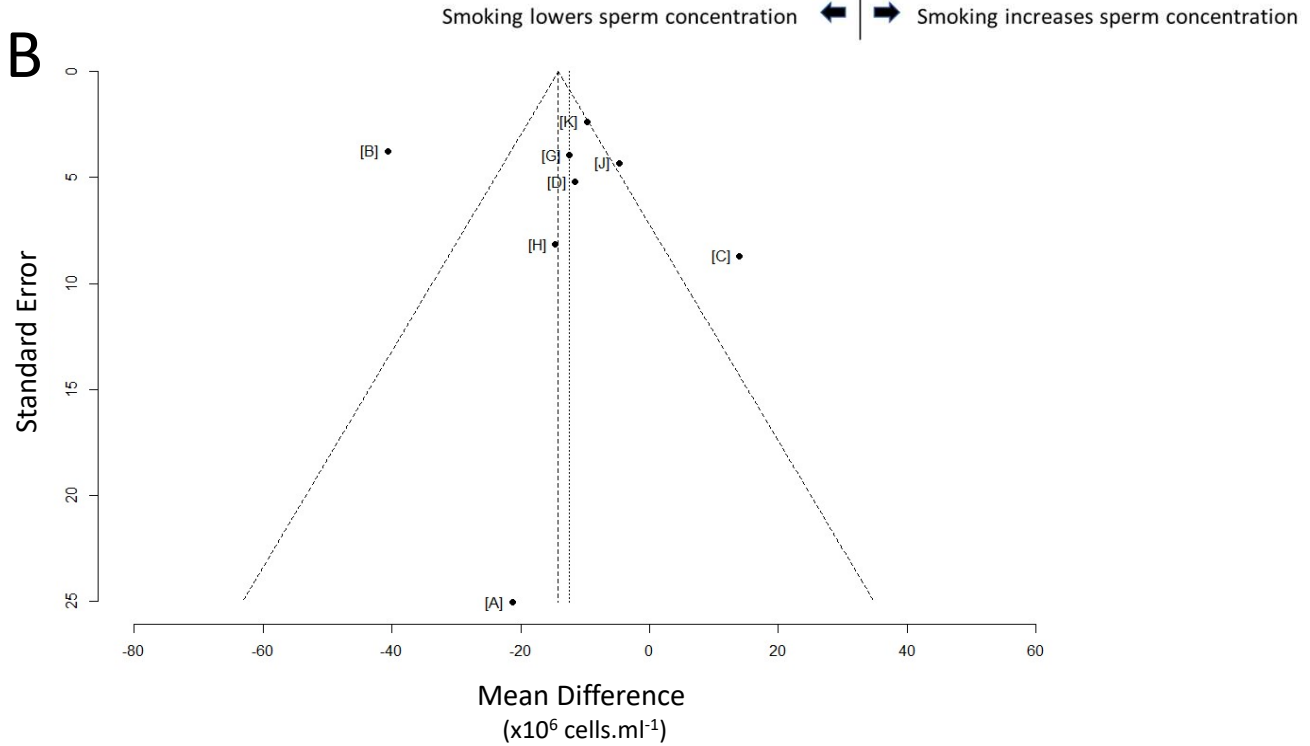
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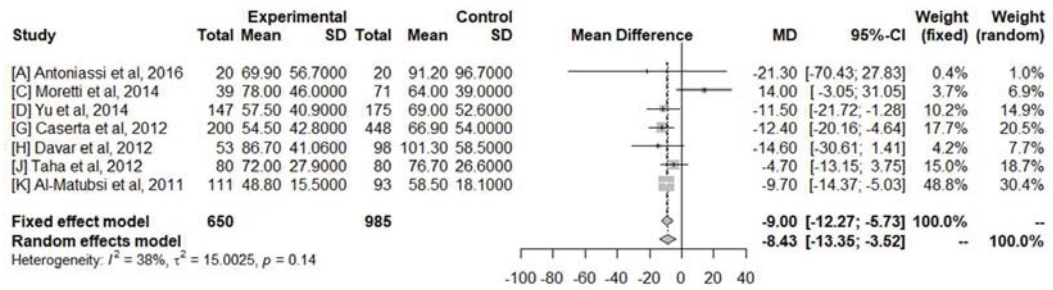
A



B



A



Smoking lowers sperm concentration ← | → Smoking increases sperm concentration

B

