META ANALYSES: BENEFITS AND PITFALLS

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INTRODUCTION

In the last 10 years there have been many research papers in the dairy nutrition area appearing using meta-analysis to analyze the data published on various subjects and about many different products. This approach has been used extensively by scientists in the medical and social professions for many years. Many recommendations are made and decisions are made about human nutrition and health based on these studies.

The central concept in the area of animal nutrition and dairy in particular is that we often have conflicting results about a nutrition principle such as fatty acid metabolism (Schmidely et al, 2008) amino acid supplementation (Patton, 2010) or a particular nutrition product like the amount of a rumen protected lysine or methionine to feed or other nutrition parameters (Hristov et al, 2005). This can be confusing. With the meta-analysis approach (Sauvant et al, 2008) it is often possible to identify those factors that are influencing the outcome which might help us to better manage the nutrition principle or use of a particular nutritional product or model.

The focus of this article will be to discuss some of the positive results that we have had from meta-analyses that have been done as well the principles that need to be followed to provide robust recommendations that we can use in the field. Also, we will discuss where meta-analysis results need to be questioned and what to look for in these results.

Why Do a Meta-Analysis

The following major reasons are why we undertake meta-analyses in preference to traditional forms of review. There may be a substantial body of literature investigating the use of a particular intervention e.g. a product or perhaps the addition of a certain feed component. By doing a quantitative analysis we can obtain an estimate of; average effect, a distribution around that effect, and, evaluate the variability or consistency in responses to the intervention. In practical terms we can provide the dairymen or ranchers a much better estimate of the likely overall effect of a treatment and some sort of confidence interval around that treatment from doing this sort of assessment. We can also indicate to them how likely they are to see a response based on the variability of the meta-analysis results.

In assessing the sources of variation, we might also be able to identify through meta-analysis some of the different factors that may influence the results and be able to advise better under what circumstances or in which populations to use our intervention. We can also look at the sources of variation and try to understand them in terms of

factors that may not have been looked at before. For example, it was possible in one study (Lean et al 2012) to have identified why fertility responses to protein interventions were variable and to identify that the effect of protein on fertility was attributable to the soluble protein component of the diet. This finding was consistent with more basic physiological studies conducted by Butler (1998) in small numbers of cattle, thereby providing strong evidence of effect and magnitude of the effect in a much larger population.

In summary, we are able to provide the producer with a better estimate of effect of an intervention, give them confidence around that estimate, let them possibly know which populations and how to best apply an intervention and lastly we can ask research questions that have not been previously answered and allow us to ask new research questions based on the results.

META-ANALYSIS PRINCIPLES AND TOOLS

Below (Table 1) are the classical tools and other approaches that are commonly used in meta-analysis that estimate the effect of interventions, quantitate the variation in effect and the probable outcome measures. Other describes some of the methods often used that can be characterized as meta-analysis (Sauvant et al (2008).

The Odds Ratio also known as the cross-product ratio is an estimator of the relative risk. It is often used in cross-sectional studies with dichotomous outcomes (e.g. pregnant or not).

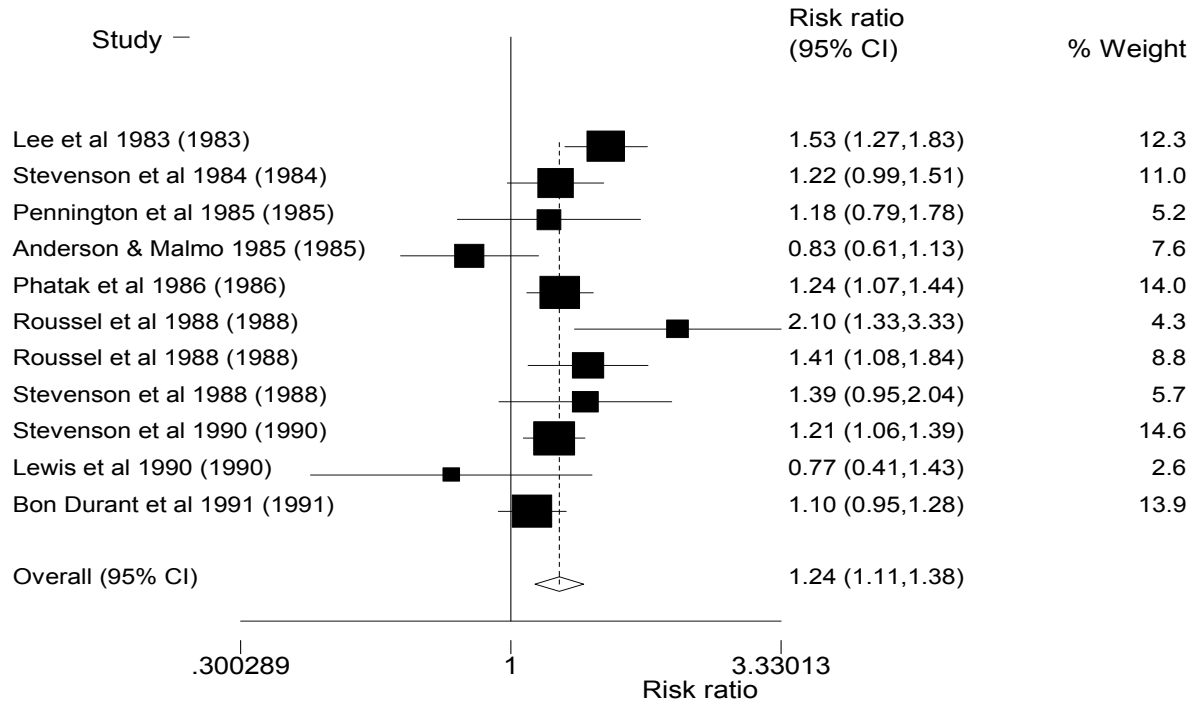
There are two plots that we commonly see in a meta-analysis, the Forest plot and the Funnel plot. A Forest plot (Figure 1) is shown below using data from a study of the use of gonadotrophin releasing hormone (GnRH) on pregnancy (Morgan and Lean 1993). This plot incorporates several dimensions and is quite powerful in describing the studies used. The X axis is the risk ratio (RR) or the increased probability of the cows becoming pregnant or not if exposed to Gonadotrophin. The left vertical line is 1.0 and denotes no effect. On the left side is list of the studies used in the analysis, usually in chronological order. The squares are the studies and the size of the squares give the reader an understanding of the size of the study and the contribution to the analyses. These figures are reinforced by the column on the right providing the weight of the study in the overall estimate. The horizontal line through the square is the confidence interval. If this line crosses the RR of 1.0, it can be inferred that there was not a significant difference for the intervention being studied. The diamond is the weighted RR, which in the case below is 1.4. The tips on the diamond represent the confidence interval for the study. In this case, the overall estimate is for an increased risk of pregnancy of 24% with a 95% confidence interval of 1.11 to 1.38 or an 11 to 38% increased risk of pregnancy.

Table 1. Meta-analysis: Positive and Negatives attributes of classical and other meta-analysis.

Positive Attributes	Classical	Other
A clear hypothesis	√	√
Comprehensive literature search at least three databases	√	√
Cleary defined criteria for inclusion or exclusion	√	√
Tables detailing studies extracted and data obtained from these	√	√
Measures of outcome analysed	Odds Ratios (OR), Relative Risk (RR), Continuous variables, hazard ratios (from survival analysis), incidence rates (disease incidence over time)	*Continuous variables Can be extended to logistic regression methods*
Weighting of Studies	Weighting based on the study variance	*Weighting best based on the study variance.*
Outcomes	Pooled OR, RR, Standard mean difference, meta regression, confidence intervals	*Weighted. Regression Means, Confidence intervals*
Estimates of heterogeneity (Sources of variation in studies)	√	√
Examination of heterogeneity	√	√
Sensitivity analysis of powerful studies	√	√
Publication bias	Funnel plots	

Generally, in nutrition we are working with continuous outcomes; is milk yield or milk protein increased? Figure 2 below is from Lean and Rabiee (2010). This Forest plot provides standardized mean difference (SMD) and their 95% CI and weights for individual trials determined from the results of 11 comparisons of milk production of cows supplemented with biotin with controls. Box sizes are proportional to the inverse variance of the estimates, so that more precise estimates have a larger box.

Figure 1. Forest plot of the Risk Ratio (RR) and their 95% CI and weights for individual trials determined from the results of 11 trials evaluating the effect of gonadotrophin releasing hormone (GnRH) on the risk ratio for pregnancy in repeat breeder cows.

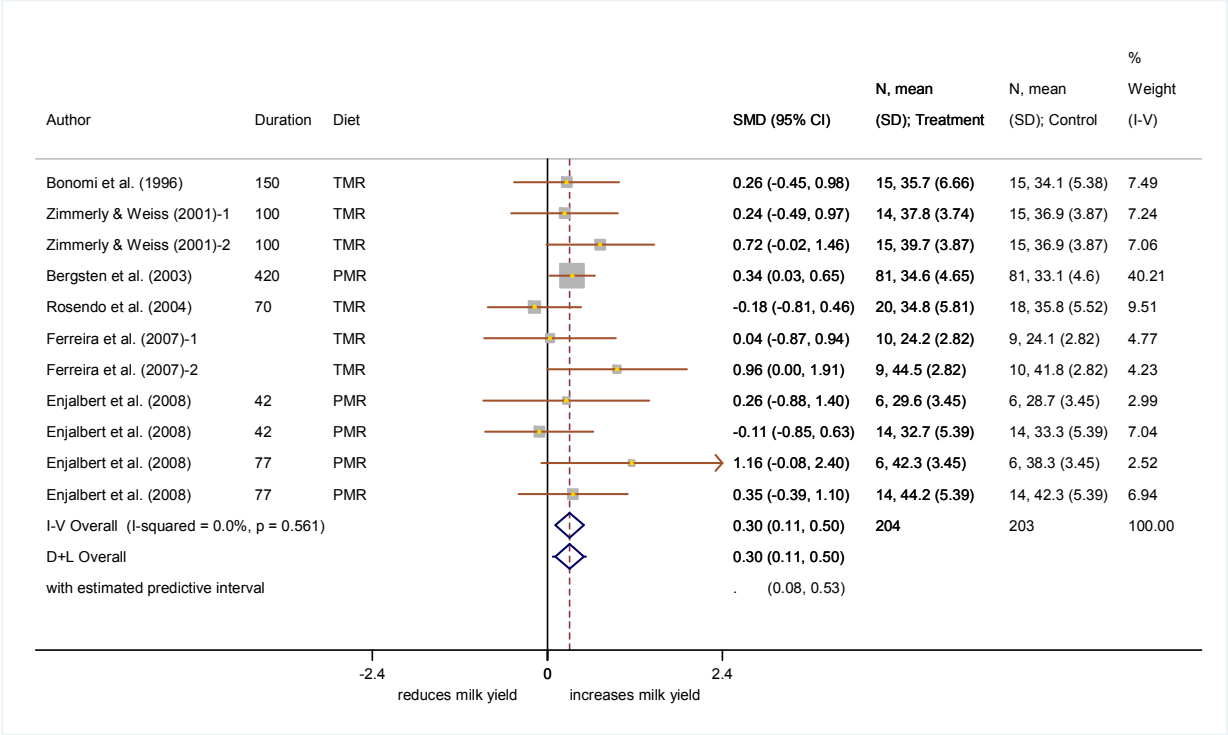


Summary estimates of treatment effects (diamond shapes) are shown using 1) a fixed effects approach (I-V specifies a fixed effect model using the inverse variance method), 2) a random effects approach (D+L specifies a random effects model using the method of DerSimonian and Laird (1986)), and 3) the predicted interval of a future trial, with the estimate of heterogeneity being taken from the inverse variance fixed effect model. The latter provides a more conservative estimate of the outcome, as indicated by the larger confidence interval. Of these measures, the most useful estimate is the random effects estimate, as it appropriately contains the random effect of trial and is a little less conservative.

The SMD measure will not be familiar to most, but is an important estimate of effect. The SMD is defined as the difference in the experimental group mean from the control group mean divided by the pooled standard deviation of the groups. The SMD, therefore accounts for study size and variance and can be used to pool different measurement scales for the same measure (a practical example might be milk weight (Kg) and milk volume (L) responses). It can be converted from the SMD, which is a z-score, if you know the standard deviation of the population. For example, if the standard deviation in weight of calves in a population is 10 kg and product x increased performance with an SMD of 0.5, you can estimate the effect will be 10 x 0.5 = 5 Kg. An alternative method, if all the measures are on the same scale, is to provide a weighted mean, based on the

inverse of the study variance, but the weighting is different to the SMD and may be less robust.

Figure 2. Forest plot of standardized mean difference (SMD) (and their 95% CI and weights for individual trials) determined from the results of 11 comparisons of milk production of cows supplemented with biotin with other cows. Note the estimate of I^2 in the third line up from the bottom. The I^2 statistic indicates how consistent (homogenous) or inconsistent (heterogeneous) studies are.



The other plot commonly used is the Funnel plot as shown below (Figure 3). This is an unpublished funnel plot from the monensin study of Duffield et al (2008) examining the effects of monensin on milk production. The funnel plot is used to examine publication bias and uses the assumption that the largest studies will be near the average effect and the small studies will spread on both sides of the average effect. Variation from this can indicate publication bias. Figure 3 depicts a symmetric distribution and also shows that the largest studies lie closest to the true effect. There is no evidence of publication bias.

If, however the funnel plot is asymmetric, it suggests that there is relationship between study size and treatment effect. In Figure 4, also unpublished from Duffield et al (2008), there is an asymmetry as small studies that might show a positive effect of monensin on milk fat are missing. We can either conclude that these do not exist or perhaps that these findings were not published. If these findings are not published, then this represents a bias in the literature, the so-called 'top-drawer' bias.

Figure 3. A Begg's funnel plot of the standard error of the SMD on the X axis against the SMD on the Y axis for the effect of monensin on milk yield. Larger studies are indicated by the larger circles.

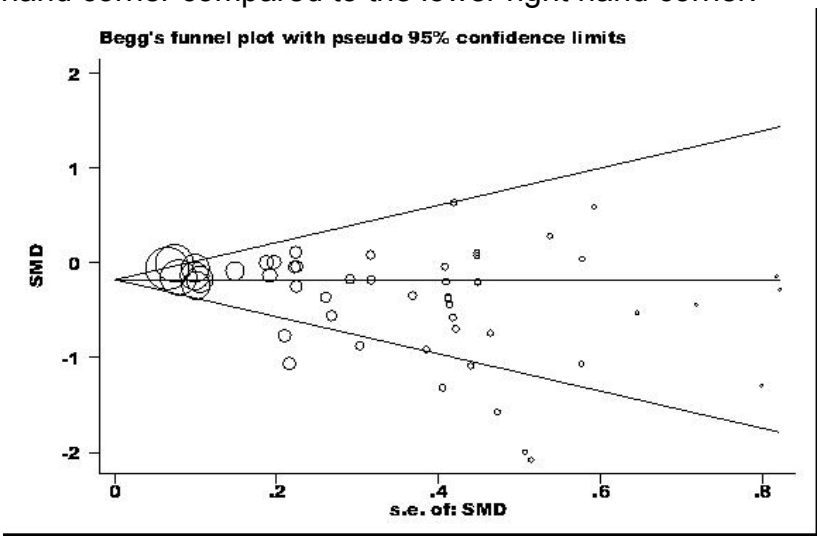
Funnel plots provide us with a good visual assessment and are particularly important where many of the studies that we conduct in our area can be small and show a non-significant effect, leading to publication bias.

This leads us to discuss the selection of studies with which to perform the meta-analysis. This is often difficult in the nutrition area because so many studies are reversals or Latin Squares. While these studies are extremely appropriate to test some hypotheses, they can have a smaller variance than randomized controlled studies, because, in part, of the 'steady state' requirements of Latin squares. This smaller variance may over-weight the value of these studies compared to longer term randomized controlled studies that may be started in the early lactation and dry periods. Many times we desire to look at the longer time effects over a lactation, such as described in the meta-analysis of Martin and Sauvant, 2002, where they looked some of the kinetics associated by the different stages of lactation.

The selection of appropriate studies dictates that a clear hypothesis be stated and that a well-defined protocol be developed for the selection of studies to be included in the analyses (St-Pierre, 2001). It is easy for studies in the dairy area to restrict ourselves to the J Dairy Sci. However this approach could very well reduce the diversity of the responses for the intervention of interest relative to the potential diversity of rations being fed (corn and soy vs. barley and canola for example). Of course if the protocol states the objective is to only look at studies then of course one would exclude studies using canola and barley. There are now electronic databases that allow for broad searches (Lean et al, 2009). The review by Lean et al., (2009) describes in detail the bases for selection. There is a concern that many studies with negative results may be rejected by reviewers yet had robust experimental designs with few biases or confounding. What can be discouraging is that one might identify 100 studies that fit the

protocol but end up with only 30 to 40 that are valid studies. The flow below gives us a visual approach to the process.

Figure 4. A Begg's funnel plot of the standard error of the SMD on the X axis against the SMD on the Y axis for the effect of monensin on milk fat percentage. Larger studies are indicated by the larger circles. Note the lack studies in the upper right hand corner compared to the lower right hand corner.



The key part in Figure 5 is the post analysis evaluation using appropriate tests for model validity and then packaging the findings in a manner that can be used with confidence in the field. This means appropriate recommendations for inclusion of the intervention in different models and platforms and then firm recommendations as to the proper places to be used in the herd in terms of use for replacements, dry cows (early, close-up or both) and lactating cows (with definition as to appropriate stages of lactation) or even in older cows or younger cows.

A suggested approach that several have been using lately in nutrition studies is to place the diets as described into either NRC 2001 or CNCPS and the variations of these models in the nutrition platforms being used. This forces the person doing the meta-analysis to be quantitative in assessing the rigor of the design and the description of the rations being fed and the analyses of the forages used in the study. So often there is an inadequate description of the forages used and the analyses of the forages. The researcher then is forced to either use book values or to discard the studies. The use of book values too often adds to the variance in adequately describing the responses.

In another dimension, especially if we can incorporate the results from many nutrition studies, we have the opportunity to look at the interactions of different nutrient levels and the intervention that is being used. For example, the intervention might be a yeast product such as the Diamond V Yeast (Poppy et al, 2012). A meta-analysis was conducted and the conclusions were; i) that there was heterogeneity in the data and ii) that there was a response in milk, fat and protein with the inclusion of yeast. The way that this heterogeneity is measured is by I^2. The I^2 test is derived from the Chi-squared

goodness of fit test that assesses the difference between observed and expected responses. An I^2 of 0 to 30 is generally considered to be homogenous (the P-value is not significant), 30 to 50 relatively homogenous (although the P value may be significant) and > 50 is heterogeneous. There are many reasons for heterogeneity in study results including dose of the product, refinement of product, feed or product delivery system, diet structure, genotype of cattle, environment and many others that influence responses.

Figure 5. Schematic representation of the meta-analytic process

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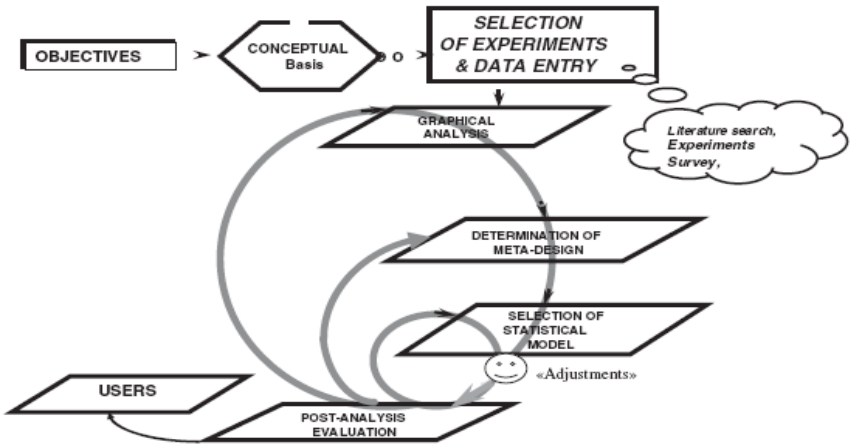

Now if one looks carefully at the data presented there was a range of responses, including no response. The question that might be asked is there an opportunity to better understand the biology by delving into the nature of the rations that were fed in the different studies? This could be a further step once one has conducted a valid meta-analysis. It is important that we know across all types of dietary and environmental situations that an intervention such as the inclusion of yeast in the ration is a worthwhile investment to make. Understand that this approach allows us to assess the probability that an inclusion is worthwhile. We can place an economic value on that risk. In this study (Poppy et al., 2012) comment was made about the possibility of other dietary effects. One could for example look at the variation in the predicted fermentable CHO fractions in the rations or the availability of adequate ammonia or degradable non-ammonia protein fractions. This would give us insight into some of the dietary factors affecting the responses that a field nutritionist could assess when adding yeast to the ration. There might also be an opportunity to be more definitive on the amount of yeast that should be included in a particular ration.

There are recent examples of how powerful the investigation of the ration and other factors influencing responses in a meta-analysis can be. This type of investigation which involves undertaking a regression evaluation on the effect size is called meta-regression. When Lean et al., (2012) examined the relationship between protein and fertility of dairy cattle, they noted the potential for confounding to arise in evaluating the interaction between dietary CP and reproduction in dairy cattle, either through inclusion

of soybean products or through changes in carbohydrate, fat or mineral content, when experimental diets were altered to increase their CP content or degradability. The hypothesis that changes in carbohydrate, fat or mineral content caused by changes when experimental diets were altered to increase CP content, influenced fertility responses had not been previously examined. Similarly, there was no previous quantitative examination of the role of soybean products and the potential of these to confound fertility responses in dairy cattle exposed to diets of higher CP content or ruminal degradability. They (Lean et al., 2012) found that all of the reduction of fertility of dairy cattle could be explained by the soluble protein of the diet and that diets containing soybean products did not influence fertility. Neither of these hypotheses could have been tested in a single comparative study. While this study used meta-regression to answer some questions, a study of milk production responses to different fats (Rabiee et al., 2012) answered some questions well, but also posed several questions in regard to the meta-regressions results. For example, what is the role of C18:0 availability at the duodenal level on milk fat production? Is it truly negative, as indicated by the meta-regression and why does magnesium percentage similarly have a negative effect on milk fat production? These questions are potentially important as they open new lines of enquiry into responses achieved in the field. The latter role of identifying new research questions is one of the most important roles of a good meta-analysis. The approach used to meta-analysis by Sauvant et al., (2008) allows an examination of heterogeneity through use of covariates in the analysis. Another way to detect sources of heterogeneity is to undertake a sensitivity analysis excluding studies that have certain characteristics. Poppy et al (2012) examined the differences between published and unpublished studies using this approach.

Below (Table 2) is a check list of the things that one should look for in a meta-analysis. With these components in the analysis, one can then provide recommendations for the inclusion of the intervention. It needs to be recognized that if there is a demonstration of significant heterogeneity in the analyses that there is the possibility that there could be other nutrient parameters that could influence the response to the intervention. A simple example (Patton, 2010) might be that one might formulate a ration to the recommended metabolizable Methionine or lysine level in the ration using an RPMet or RPLys source and see no response. This could be because with the ingredients being used Methionine or Lysine is not first limiting or things like an overestimate of metabolizable Methionine or Lysine from microbial contributions or an over estimate of the metabolizable Methionine or Lysine from the ingredients being fed.

SUMMARY

Meta-analysis can become a powerful tool, if done properly. It helps us sort out the sometimes confusing published results from different studies to provide us with an increased degree of certainty if an intervention will work. This approach will also give us a confidence range in which the intervention will work and the risks associated with this intervention. One can with this approach apply economics and assess the probable return on investment in applying the intervention. Additionally, with the use of meta-regression along with classical meta-analysis we can identify the interactions of

Table 2. A check list of things to look for in a meta-analysis

Attribute	**Positive aspects**
Literature search	Three or more search engines, well described key words, inclusion criteria well described, exclusions clearly detailed,
Hypothesis	Well-structured and clearly stated
Statistical Analysis	Appropriate to the data
Estimates of effect	Significance of treatment effect, confidence intervals, fixed or random-effects models
Examination of heterogeneity	Using Chi-square, I^2, others
Evaluation of heterogeneity	Sensitivity analysis, meta-regression, covariate evaluation
Evaluation of publication bias	Funnel plots

changes in management, nutrient sources, etc. with the different levels of the intervention. This can be of great value as we formulate rations for dairies being able to apply nutrient and management constraints in the formulations. One added benefit which we do not think about is that with the meta-analysis procedure there might be an initial selection of 100 potential studies. Many of these studies are rejected, because there is inadequate nutritional information in the studies. This should be a heads up to scientists and to reviewers of the importance of providing essential information in describing the studies conducted. It is much better now than in the past. Additionally if it is demonstrated that there is significant publication bias this is also a heads up to scientists and reviewers that if the study is well conducted with no or negative results that these studies should still be considered for publication.

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