A113 On the Central Importance of Analysis of Covariance (ANCOVA) in Human Skeletal Research

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After attending this presentation, attendees will understand: (1) the power and flexibility of ANCOVA and the important role it can play in interpreting skeletal variability; (2) how basic errors in experimental design severely limit the utility of many forensic osteology studies; and, (3) how the choice of statistical procedures can lead to an over-emphasis on subgroup differences.

This presentation will impact the forensic science community by providing a new "best practice" standard for skeletal research that focuses on completing rigorous multivariate hypothesis testing before generating predictive equations.

The ability to construct a biological profile for the skeleton of an unidentified decedent is predicated on the availability of reliable comparative data, and the accuracy of that profile will vary directly with the quality and depth of prior skeletal research; however, a review of research published in the *Journal of Forensic Sciences* during the last two decades indicates that most studies suffer from one or more major flaws that limit their applicability in forensic settings. These flaws include errors of experimental design and inappropriate or incomplete statistical testing.

The following errors of experimental design were noted: (1) truncating or completely omitting fundamental hypothesis testing procedures. Many studies fail to properly isolate all relevant independent variables (sex, ancestry, body size, age at death) and thus cannot determine which ones actually affect the variance in a skeletal feature; (2) generating predictive regression or discriminant equations for subgroups based on variables that have no proven impact. If an independent variable is not significant in the variance equation, then there is no cause to generate separate equations for those subgroups. For example, stature equations are often provided for sex and ancestry subgroups without demonstrating that they substantively reduce estimation error over mixed-group equations; and, (3) inadequate sampling for the number of variables examined. To thoroughly test hypotheses, large samples are necessary because the addition of each new independent variable splits the sample across an increasing number of cells. For example, 200 specimens used in a typical 28-cell design (two sex groups vs. two ancestry groups spanning seven age decades) results in fewer than eight individuals per subgroup, reducing the power of subsequent statistical testing.

The following problems with statistical testing were noted: (1) over-reliance on simple univariate and bivariate procedures. Chi-squared tests, *t*-tests, and correlation cannot simultaneously control for the effects of multiple independent variables, and so the apparent effects of one variable may be due to the (masked) effects of a second (uncontrolled) variable; (2) ignoring interactions. Simple tests are susceptible to the confounding effects of interactions, which occur when the combination of two variables produces an added effect that cannot be attributed to either variable individually; (3) ignoring unbalanced sampling. Simple tests are influenced by uneven sampling, and since few death assemblages are balanced, most studies must overtly compensate; and, (4) assuming that other complex procedures actually test hypotheses. Principal components analysis and regression, for example, are not adequate for basic hypothesis testing.

A solution to these various problems is to employ ANCOVA as a first step in the experimental process. ANCOVA is a robust and sensitive hypothesis-testing procedure that partitions the variance across all independent variables simultaneously, separating the effects of interactions and controlling for unbalanced samples. The independent variables can be categorical (sex, ancestry) or continuous (body weight, age at death), and varieties of ANCOVA exist for categorical dependent variables (binary logistic regression, ordinal regression). Independent variables that are flagged by ANCOVA as significantly affecting the variance in a skeletal feature can then be explored further to construct predictive models for use in forensic settings.

The historical tendency to eschew thorough hypothesis testing and instead rely on simple statistical tests can explain many contradictory findings between different studies of the same skeletal feature. Furthermore, over time, these errors may have led researchers to emphasize the importance of subgroup differences (and thus the necessity of population-specific methods) at the expense of more robust, non-specific techniques with wider applicability. Until multivariate approaches such as ANCOVA are consistently applied, we will not be able to assess the true importance and relevance of subgroup differences in human skeletal variability and their practical effects on biological profile construction.

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