population from the observations recorded in the sample studied. These observations are summarized in a manner that describes the central tendency of the data and their variability. These summary measures provide estimates of the true (but unknown) value in the population. Because it is usually not possible to study the entire population, the sample chosen should be representative of the population so that the results obtained can allow one to make inferences about the true effect in the population. Such inferences rely on a probabilistic approach whereby one can quantify the degree of uncertainty associated with the inductive inference. Ideally, the sample is chosen by a random process so that selection bias is avoided. Each subject in the sample is picked only once and contributes data that are specific to that individual.

In the ovulation induction literature, the samples that have been studied have usually been composed of subjects who contributed one or more cycles of data. This approach is considered by the investigators to be acceptable because the "sample size" becomes large very quickly. Unfortunately, this approach is flawed for many reasons. If the objective is to identify predictors of multiple pregnancy, each subject should only contribute data from one cycle. In this manner, meaningful descriptive statistics can be generated and appropriate methods can be applied to analyze the data. For example, if female age is an important prognostic variable, each subject independently contributes one data point for this variable, which can be correlated with the outcome, and summary statistics (such as mean and variance) have more meaning and can be compared statistically. When data are derived from more than one cycle per subject, a problem of lack of independence is created.

Thus, a woman who is 32 years of age and undergoes three cycles of treatment within 4 to 6 months contributes three sets of data. The current (incorrect) approach is to associate each set of such data with female age of 32 years. But in doing so, the subject in the sample is used three times (scenario 1). The inference from such data about the population is not the same as if three different subjects 32 years of age were studied in one cycle each in the sample (scenario 2) or if a different subject 32 years of age was studied for one cycle in each of three different samples (scenario 3).

The estimate produced in the third scenario gives an indication of the effect of sampling variability on the true estimate. The second scenario gives an indication of between subject variability. Both scenarios are of value because they gather observations from subjects who are independent of each other.

In contrast, the first scenario (as used by Dr. Dickey and other investigators) provides data that are not independent of each other and give only an indication of intrasubject variability for outcome events that are not terminal (e.g., E_2 level or number of follicles). Furthermore, the treatment regimen used in a second or subsequent cycle is often influenced by the performance in the earlier cycles, thereby leading to biased assessments of outcomes. Also, only subjects who do not conceive in the first cycle undergo another cycle of treatment. Consequently, subjects who conceive in the first cycle may belong to a different prognostic category than do those who are undergoing treatment in their fourth or fifth cycle. These problems of lack of independence, bias, and heterogeneity make it difficult to accept the results of the cohort studies to which Dr. Dickey refers.

The use of logistic regression is appropriate and valuable to identify the variables that best predict multiple pregnancy. However, to do so requires data from individual (independent) subjects. When several cycles from one subject are used, the variable in question (e.g., female age) has more influence producing bias that is reflective of the number of times it is recorded in the same subject's data sets.

In summary, the inferences made from the published cohort studies to which Dr. Dickey refers are biased because of lack of independence. The ideal approach would be to only analyze data from one cycle (preferably the first cycle) per subject. The results of such analyses would have more relevance in identifying the predictors of multiple pregnancy so that strategies can be put forward for measures to reduce the frequency of this outcome event. Until then, the Educational Bulletin issued by the American Society for Reproductive Medicine (2) is still valid in its conclusion that ideal threshold criteria for hCG administration to reduce the incidence of multiple pregnancy cannot be identified with confidence from the current literature.

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Polycystic ovarian disease and serum leptin levels?

To the Editor:

Remsberg et al. (1) recently found that serum leptin levels did not differ among women with the polycystic ovary syndrome (PCOS) and age- and body mass index (BMI)–matched controls. But when they separated patients into tertiles, they observed that women with PCOS below a certain threshold BMI had lower leptin levels than did controls. They concluded that below a certain BMI, hyperan-

drogenic women with PCOS have lower leptin levels than controls.

Insulin has been shown to have direct regulatory effects on gene expression and leptin production. Conversely, androgens have been reported to have a suppressive effect on circulating leptin concentrations. Remsberg et al. (1) concluded that women with PCOS appear to produce insufficient leptin relative to the degree of hyperinsulinemia, potentially because of the competing effects of adipocyte insulin resistance and androgens on leptin. They found low leptin levels only among women with PCOS in the lowest BMI tertile.

Numerous studies have explored the serum leptin levels in women with PCOS. Mostly, it has been found that patients with PCOS have higher leptin levels than controls (2). Patients with PCOS are usually obese, and the alteration in leptin levels usually correlate with BMI (3). In contrast to Remsberg et al.'s (1) results, Brzechffa et al. (2) reported that serum leptin levels were positively correlated with serum free T levels. Rouru et al. (4) found a positive correlation with serum free T and leptin levels in patients with PCOS, although the effect disappeared after partial regression analysis.

We investigated serum leptin concentrations in obese (n = 12) and lean (n = 22) patients with PCOS. Our aim was to examine serum leptin concentrations in obese and lean patients with PCOS to assess whether the changes in leptin levels are due to obesity or hormonal alterations. We found a significant correlation between serum leptin concentrations and obesity variables as determined by body composition measurements or dual-energy X-ray absorptiometry. Insulin resistance showed significant correlation with serum leptin levels. However, when the BMI effect was excluded by partial correlation analysis, these correlations disappeared. Hyperandrogenemia was not correlated with serum leptin levels. When we separated patients according to serum androgen levels, serum leptin levels also did not differ significantly between groups. We concluded that insulin-stimulated glucose transport is diminished in adipocytes of women with PCOS independent of obesity.

Leptin deficiency and polycystic ovary syndrome appear to be similar entities, with obesity, irregular cycles, and hyperandrogenism. Hyperandrogenemia, insulin resistance, and hyperinsulinemia may also affect serum leptin levels.

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Reply of the Authors:

Erturk and Tuncel responded to our article (1) by presenting their data on leptin, insulin, and androgens in 12 obese and 22 lean women with the polycystic ovary syndrome (PCOS). They found that leptin levels are positively correlated with obesity and insulin resistance and not correlated with androgen levels. After adjusting for the effect of obesity, insulin resistance was no longer an independent predictor of leptin.

We examined leptin, insulin, and androgens in women with PCOS and normally cycling weight-matched controls. In contrast to other studies of leptin and PCOS, we stratified these women by tertiles of body mass index (BMI) to determine whether the relationship between leptin and BMI in PCOS was consistent across normal weight, overweight, and obese subgroups. As in other studies of leptin, we found an overall nonsignificant difference in leptin levels between women with PCOS and normally menstruating women (1, 2), as well as an overall positive association between leptin and body fat, androgens, and insulin. However, after adjustment for BMI, we observed a negative but statistically nonsignificant correlation between total T and leptin ($r = -0.124$; $P=.275$), which supports the inverse relationship reported by Carmina et al. (2). Among women with BMI of 25 kg/m^2 or less, the leptin level was significantly lower in those with PCOS than controls; in the highest BMI group, leptin levels were nonsignificantly increased in women with PCOS.

As stated in our article, these findings support the theory that normal-weight women with PCOS may have an underlying leptin deficiency related to androgen excess. Given the degree of glucose intolerance and insulin resistance observed in our obese participants with PCOS compared with BMI-matched controls, we suggest that obese women with PCOS may also have an underlying deficit of leptin production or, alternatively, leptin resistance relative to body size and fat mass. This observation remains consistent with a potential competing negative influence of androgens on circulating leptin concentrations.

From this observational study alone, it is not possible to detect the full range of leptin signaling action. For example,