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Reply of the Authors:

We thank Professor Sismondi and associates for their most interesting letter. We appreciate the additional information based on their current research on potential mechanisms whereby certain progestins may cause breast cancer, in conjunction with or independent of estrogens. Such basic research is crucial not only for deciphering the sometimes confusing results of previous epidemiologic studies but for aiding in the optimal design of future studies. For example, if an intermediate growth factor or hormone were implicated in a causal pathway (such as sex hormone-binding globulin, serum insulin-like growth factor I, or serum growth hormone as discussed by Sismondi and associates), then measurement of this factor directly, in addition to exogenous hormone dose, would be necessary to explore this relationship in large populations.

We would also like to thank Dr. Edgren for his response to our review, as it addresses what we believe to be one of the key methodological issues in studying the relationship between progestins, as a therapeutic class, and breast cancer. However, we would like to take this opportunity to clarify two comments made by him.

First, Dr. Edgren asserts that we have "continue[d] the now discredited concept that ethynodiol diacetate, the progestagen in Demulen, is a high potency progestagen." Although we did describe two epidemiologic studies that both classified ethynodiol diacetate as a high potency progestin and found an association between products containing this drug and breast cancer (1, 2), we did not intentionally infer support of this concept; rather, we presented the classification of progestins as a major weakness of the Pike study. Indeed, in our introductory section on "Progestins," we discussed the different types of potency testing and the fact that potencies of progestins vary by type of potency studied, the test used to determine potency, the species of animal tested, and the presence or absence of estrogens (3). In this discussion, we tried to convey the view that the methods used to determine potency are not without variation or controversy; a point also made very clearly by Dr. Edgren.

Second, Dr. Edgren states that we advocate the use of single numbers for potency of combination oral contraceptives. Again, because our purpose was to review previous studies, we described studies where such classification was used and briefly discussed the controversy surrounding the determination of relative potencies of progestins, with and without progestins. An in-depth discussion, we felt, was beyond the scope of our paper, but an excellent review of this topic is available, as was indicated by Dr. Edgren (4). We agree with Dr. Edgren's viewpoint that each progestin and progestin-estrogen combination should ideally be considered as a distinct exposure in epidemiologic studies and further point out the need for baseline information regarding endogenous hormone levels before drug administration to further understand varying impact of that exposure between individuals.

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Levitating Human Sperm—An Adman's Dream

To the Editor (Letter 1 of 2):

We would like to refer to the article by Blanchet et al. (1). As the group who pioneered laser micromanipulation of gametes, we are pleased to see more studies that might elucidate the complexity of light

and gamete interaction. However, several comments are needed to clarify the physical properties of the laser system described. In characterizing a laser interaction, one should be keenly aware of the tremendous versatility of lasers and the large number of possible parameters, each, in turn, may result in significantly different effects on the target area. In their article, the authors describe only a single combination of laser parameters (i.e., single wavelength, pulse duration, and energy per pulse). The specification of the pulse repetition rate (PRR) is missing. According to our experience, a high value of PRR can lead to excessive localized heating and oocyte mortality. Furthermore, the authors reviewed the literature on laser application in various areas, but ignored previous applications of lasers (in a large variety of wavelengths) for gamete micromanipulation. The potential use of these accurate beams for gamete manipulations was first described in 1989 (2). In this set of experiments, oocytes were manipulated with laser beams at the ultraviolet and visible wavelength range for zona drilling and inactivation of an extra pronucleus after polyspermic fertilization, as well as trapping sperm with a laser-generated optical trap at the near infrared range. This unit was further developed into a device that measures the relative force of single spermatozoa (3). A comprehensive review on these modalities was published in 1991 (4). Later on, Palanker et al. (5) have used the argon fluoride excimer laser (197 nm) to drill the zona pellucida of mouse oocytes, and after insemination they were fertilized and cleaved to the blastocyst stage. After their date of submission, several other articles have been published on micromanipulation of human sperm and oocytes. The article by Blanchet et al. adds another valuable piece of information to this new field. However, due to the close proximity of the wavelength used (KrF at 248 nm) to the DNA absorption peak at 260 nm, the question regarding the safety of this particular laser must be answered before such a system can be considered for clinical applications.

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Note. Additional references are available from author upon request.

Reply of the Authors:

As stated in our publication, the zona pellucida was perforated using two to three laser pulses. The interval between laser pulses was 3 to 5 seconds, assuring that no displacement of the oocyte had occurred between successive pulses. Estimates of thermal time constants using heat conduction only and ignoring the additional cooling due to convection processes indicate that this lengthy time delay is clearly sufficient to alleviate any concerns about zona heating effects. It was also stated in the article that laser fluences ranging from 0.6 to 3.0 J/cm^2 were acceptable for zona pellucida perforation. Results were reported at 1 J/cm^2. Lower fluence levels required numerous pulses to etch through the zona pellucida, whereas higher fluences offered no significant advantage, also increasing the risk of embryo damage.

With regard to additional references, it was never our intention to provide a review of all work in the quickly burgeoning field of laser micromanipulation. Clearly there are a large number of important contributions from a wide range of authors. It was not our intent to slight any of these but only to present the material on which we based our experiment. If the group of authors feel slighted, then we apologize as we recognize the importance of their contributions and wish them much success in the future.

Although there is no implicit or explicit mention of clinical trials in our work, it is abundantly clear that, as for all new techniques, considerable work will be necessary in preparation for clinical trials. The purpose of our article was only to report the results of an experiment that might be of interest

to the scientific community. Finally, we might add that if there were considerable genetic damage induced by the particular laser wavelength, one might expect, contrary to our observation, high mortality rates due to unselective damage.

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To the Editor (Letter 2 of 2):

We read with great interest the article by Strohmer and Feichtinger (1) that describes a successful clinical application of laser for micromanipulation in an in vitro fertilization (IVF) program. In their discussion, the authors have stated that "it would be preferable to use laser that does not require direct contact, but at the moment there is no possibility to cause photoablative effect at an appropriate wavelength by focusing an unguided microbeam." We agree that the noncontact laser microbeam is preferable for gamete manipulations. Contrary to this statement, it is important to note that these systems are available. Indeed, this was our approach when we first introduced this application in 1989 (2), and another study recently support this approach (3). This information is valuable because coincidentally, in the same issue, there was a commercial advertisement, "The IVF laser . . . your laser system for micromanipulation techniques in the IVF laboratory. . . . safe and simple," with a reference to this article.

In their article, the authors emphasize the reason for selecting a laser in the infrared range, and misquote our article (4) as if we have stated that the 366-nm laser may cause adverse effect on the genetic material. In our article (4), we described the use of four different laser wavelengths (266, 355, 366, and 532 nm) and achieved the best results with the 366 nm. It is known that the DNA absorption maximum is at 250 to 260 nm; however, at the 300-nm range the absorption is considerably less (5). In addition, the tangential noncontact delivery mode avoids direct exposure of the genetic material itself. Furthermore, in the noncontact mode, the need for some elements of the conventional micromanipulations setup is avoided. Simplifications include elimination of the need for holding vacuum pipette or reshaping and sterilizing insertion glass pipettes (or fibers). It is not common to refer to an advertisement in a letter to the editor, however, since it is stated in the same context that "safety and simplicity is proven in experimental and clinical investigations," we would like to suggest that more experimental and clinical studies of various laser parameters are needed before such statement. In our recent studies we focus on the 308-nm laser (again, in the noncontact mode) and find it superior to other lasers that we have tested previously. There is no doubt that lasers may be the most accurate and versatile tool for micromanipulation; however, this has yet to be demonstrated. Careful studies defining wavelength, delivery systems, safety, and, above all, the appropriate biological problems for this technology have yet to be conducted.

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Reply of the Authors:

We thank Dr. Tadir and colleagues for their repeated (ongoing) interest in our erbium:YAG laser system. After our first publication of successful ap-