Dear Sir,

The role of LH in folliculogenesis and oocyte maturation, and consequently the usefulness and timing of exogenous LH activity administration in controlled ovarian stimulation, has recently become a widely debated issue. During a spontaneous ovulatory cycle serum LH levels progressively increase across the follicular phase (Filicori et al., 1986). Together with the concomitant decrease in serum FSH concentration, this LH increase is believed to be responsible for the development of dominance of the leading follicle and the demise of smaller follicles that started their FSH-dependent growth at the beginning of the follicular phase; in fact the increasing levels of LH can substitute for the declining FSH only in larger follicles that have developed LH receptors in their granulosa cells but not in smaller follicles whose granulosa cells are devoid of LH receptors (Campbell et al., 1999).

If follicular-phase LH activity makes part of the physiological selection mechanism leading to follicle dominance and monofollicular ovulation, the philosophy of most ovarian stimulation protocols reported to date goes against this physiological process, in search for recovery of multiple developmentally competent oocytes. It is true that this approach can create problems, mainly related to multiple pregnancies and the development of ovarian hyperstimulation syndrome (OHSS). The cardinal question that nourishes most of the ongoing debates about LH in ovarian stimulation is whether the administration of exogenous LH activity (in the form of natural or rhLH or chorionic gonadotrophin) can lead to a better compromise between the efficacy and safety of the ovarian stimulation protocol.

Reports evaluating the effects of exogenous LH administration during ovarian stimulation on outcomes of assisted reproduction are ambiguous, as recognized both in our paper at the origin of this debate (Tesarik and Mendoza, 2002) and the comment by Filicori et al. (2003) above. This is not surprising in view of the diversity of criteria according to

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which data were evaluated in different studies and of the multiple sites of action of LH in the female reproductive system. From a purely pragmatic point of view, there appears to be little doubt that co-stimulation with exogenous LH, in addition to FSH, gives clinical pregnancy and birth rates at least equivalent to protocols using FSH alone and, at the same time it reduces the cost of the procedure and the risk of OHSS (Filioric et al., 2002). However, the effects of LH underlying these observations are not necessarily related merely to oocyte yield and developmental competence because LH exerts direct effects on the uterus as demonstrated recently by evaluating the effects of endogenous and exogenous LH activity on endometrial thickness and uterine receptivity of women lacking ovarian function and receiving embryos from an oocyte donation programme (Tesarik et al., 2003).

In view of the above considerations, the effects of exogenous LH administration during ovarian stimulation on the yield of developmentally competent oocytes is extremely difficult to evaluate objectively in standard assisted reproduction attempts in which ovarian stimulation, oocyte recovery and embryo transfer are performed in the same individual. This is the reason for which we designed a research project using an oocyte donation programme as a model with which pertinent information about the effects of LH on oocyte yield and quality can be obtained independently of eventual LH effects on the uterus. Moreover, the sensitivity of certain outcome measures, such as the implantation rate, is obviously higher in this system, in which oocytes from the same donor are shared by two different couples, as compared with models based on the transfer of all embryos to the same woman. With the same number of embryos transferred per patient, the oocyte-sharing policy enables the evaluation of the quality of a two-times higher number of oocytes recovered with the use of a given type of ovarian stimulation regimen as compared with the situation in which embryos from one ovarian stimulation attempt are transferred to one patient only. In the latter situation, the current possibilities of embryo selection may counteract the manifestation of poor quality of a part of oocytes recovered. Our study evaluating the effects of exogenous LH administration during ovarian stimulation on oocyte yield and developmental competence (Tesarik and Mendoza, 2002) was based on this reasoning.

The primary variables evaluated in the above study were the number of metaphase II oocytes, the number of normally fertilized (two-pronucleated) zygotes and the implantation rate after transfer of similar numbers of embryos developing from donated oocytes to recipients whose uterine receptivity was prepared with the use of the same protocol. Pre-study calculations to determine sample size were performed in view of these variables. The group size was sufficient to detect differences in these primary variables between cases in which exogenous LH was included in the stimulation protocol and those in which it was not. The other data reported [serum estradiol (E₂) and LH levels and the number of follicles on different days of ovarian stimulation, the peak serum E₂ level, the total dose of FSH administered during ovarian stimulation, the total number of oocytes retrieved, the numbers of normally and abnormally fertilized oocytes, morphology of zygotes and cleaving embryos, and the pregnancy rate] were considered as secondary variables. A larger sample size might be necessary to fully appreciate the impact of exogenous LH on some of these secondary variables.

The study was designed to compare outcomes of attempts in which LH was included in the ovarian stimulation protocol for oocyte donors and of those in which FSH alone was used. To reduce the impact of a particular pattern of ovarian responsiveness to stimulation, inherent in each oocyte donor, we further subdivided both groups of donors to a subgroup of higher responders (day 4 serum E₂ concentration of ≥100 pg/ml) and a subgroup of lower responders (day 4 serum E₂ concentration of <100 pg/ml). Accordingly, the most objective comparisons can be done between groups of donors with similar pre-stimulation serum LH levels and with similar early response to ovarian stimulation. Nonetheless, for the reader’s interest, P-values that are indicated in the Tables of our paper (Tesarik and Mendoza, 2002) allow formal comparison of each individual subgroup of donors with any other one. The reader is expected to consult study design, as presented schematically in Figure 2 of that paper, to make interpretations.

Selection criteria for oocyte donors were chosen to exclude women with diminished ovarian reserve and those with a high risk of OHSS. Accordingly, only women of <28 years of age, with more than 10 small antral follicles detected in the two ovaries on days 1–3 of the cycle, with the absence of the ultrasound picture of polycystic ovaries, and whose fasting glucose:insulin ratio was >4.5 were involved. In agreement with a previous report (Legro et al., 1998), this ratio has proven to be a reliable indicator of insulin resistance, and thus of an increased risk of the development of OHSS, in our oocyte donors.

The idea of searching for insulin resistance in oocyte recipients is an interesting one, and we are now testing the fasting glucose:insulin ratio in all patients involved in our oocyte donation programme. It is true that this examination was not performed in the patients enrolled in our previous study (Tesarik and Mendoza, 2002). However, there is no reason why isolated cases of insulin resistance, if present, should be non-randomly distributed between individual patient groups. Thus, the lack of the control of insulin resistance in oocyte recipients is highly unlikely to have impacted on the results of the above study. The same applies to endometriosis which also can diminish the chance of clinical pregnancy in an oocyte donation programme.

In view of all these facts, the study by Tesarik and Mendoza (2002) is by no means in contradiction with other recent studies evaluating the effects of the inclusion of exogenous LH activity to the ovarian stimulation protocol (Schoolcraft et al., 1999; Gordon et al., 2001; Ng et al., 2001). The impairment of oocyte quality in groups of donors with high endogenous LH in whom exogenous LH was administered (Tesarik and Mendoza, 2002) was partly compensated by a higher yield of oocytes in these groups. Moreover, the manifestation of the oocyte impairment was facilitated by the special character of this study design, in which oocyte sharing between two different recipients increased the sensitivity of the implantation rate by doubling the proportion of oocytes from each cohort resulting from a single ovarian stimulation attempt that were actually used for
fresh embryo transfer. This is also the main reason for which these data are not in contradiction with our earlier findings showing that high intrafollicular concentrations of LH were associated with rapid cleavage of embryos (Mendoza et al., 1999) and that the mean concentration of LH in the fluid aspirated from all follicles at the time of oocyte recovery was higher in attempts in which a clinical pregnancy was achieved as compared to unsuccessful attempts (Mendoza et al., 2002).

The mechanism of the effects of LH underlying the observations published by Tesarik and Mendoza (2002) has been suggested in the above paper and appears to be related to the opposite effects of LH at the beginning and in later phases of ovarian stimulation. At the beginning of ovarian stimulation the addition of LH can promote the recruitment of small antral follicles to the FSH-responsive pool. This effect can be explained by the stimulation by LH of androgen production by theca interna cells of small antral follicles, according to the two-cell/two-gonadotrophin model (Hillier et al., 1994). Because antral follicles at the beginning of stimulation are small and contain relatively low numbers of granulosa cells, most of this androgen is not efficiently converted to estrogen and accumulates in the ovary. Experimental studies in primates support a stimulatory role for androgens in the development of early antral follicles (Vendola et al., 1998; Weil et al., 1998), probably related to a strong stimulation of the expression of FSH receptor in granulosa cells (Weil et al., 1999). However, the accumulation of androgen is only temporary because antral follicles convert increasing amounts of androgen to $E_2$ as they grow, which leads to increasing $E_2$ concentrations in blood serum. As indicated in the Materials and methods section of our previous paper (Tesarik and Mendoza, 2002), the dose of FSH was adapted to the individual increase in serum $E_2$ and to the number and size of antral follicles in all donor groups from day 8 of ovarian stimulation, but the dose of LH was maintained stable at 75 IU/day in the groups co-stimulated with this hormone. This obviously favoured larger follicles that already have developed LH receptor in their granulosa cells.

In conclusion, our data strongly suggest that there is both a threshold and a ceiling for the LH dose with which the maximal number of developmentally competent oocytes can be obtained from a single ovarian stimulation attempt. In current practice, however, ovulation stimulation regimens must be tailored to a concrete clinical situation rather than being aimed at maximum oocyte yield. Co-stimulation with LH during the late follicular phase may be clinically advantageous even in some cases in which endogenous LH is relatively high by reducing the cost of stimulation and the risk of OHSS. On the other hand, in situations in which ovarian stimulation is performed with the maximum yield of developmentally competent oocytes as the primary goal, co-stimulation with LH should have to be adapted to the current endogenous LH level to obtain optimal outcome. Accordingly, LH can be used at the beginning of stimulation, to promote follicle recruitment, and discontinued later to optimize the chance of recovering healthy oocytes from the maximum of the follicles recruited.

References


