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OPINION

Change, change, change: hormonal actions depend on changes in blood levels

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The main hypothesis outlined in this communication is that changes in hormonal levels are of utmost importance in the female reproductive system physiology. Hormone measurements must be assessed in the context of time and change. We hypothesize that changes in hormone concentrations carry significant biological messages, much more than a given level at a given time point and if proved, this theory could give rise to better approaches to treatment, and risk assessment.

Keywords: hormones; female reproductive physiology; change; dynamics

All life forms on the face of the earth are constantly moving at high speed. Our planet circles around its axis every 24 h, and completes a huge journey in orbit around the sun every year. We do not feel this tremendous velocity, but the body does sense and respond to relatively minute changes in velocity. We are geared to detect changes.

Routine clinical work depends on hormone measurements. Do the results obtained by spot sampling really reflect the endocrine environment and action? A prominent example for a negative answer to that question is native GnRH. This hormone exerts its action by pulses, giving an extreme example that changes in blood level (hypothalamus-pituitary portal system) are far more important than the level itself at a given moment. If we cancel GnRH pulsatile secretion by administering native GnRH in a continuous rather than pulsatile fashion, or indeed its agonist, we induce down-regulation, or complete inhibition of the biological activity. It is the changes in the amplitude and frequency of GnRH pulses that largely dictates the quantity and timing of secretion of LH and FSH, so essential to produce a normal ovulatory cycle (Moenter *et al*., 1992).

Do other hormones associated with reproduction act, at least in part, by changes in their blood levels rather than absolute concentrations? To keep things simple in the clinical arena, we tend to ignore this possibility. Below we provide examples of how the changes in serum concentrations rather than absolute levels of particularly LH, but also progesterone, estradiol (E_2) and HCG influence normal physiology and, in some cases, the mode of treatment.

The purpose of the current communication is to offer a fresh look at the hormones that we measure routinely, and to inspire further research.

Luteinizing Hormone

The introduction of recombinant FSH in the mid-90s ignited a debate on whether ovarian stimulation can depend on FSH only. This debate took a fresh turn when GnRH antagonists were introduced. Many studies tried to look at the effect of LH levels at certain time points during stimulation (mostly on the day of ovulation trigger with HCG) on cycle outcome (reviewed by Alviggi *et al*., 2006; Griesinger and Diedrich, 2006; Kolibianakis *et al*., 2006; Kolibianakis *et al*., 2007). These reviews could not establish any basis for adding LH during stimulation, yet, not uncommonly in our experience as overseers of artificial reproduction treatments (ARTs), clinicians change to urinary gonadotrophins (containing LH activity) or add recombinant LH when the antagonist is introduced into the stimulation scheme.

Previously, it was suggested that adding LH may prove beneficial to those patients who hyper-respond to the antagonist by a sharp drop in LH levels. Since the antagonist daily dose is fixed (0.25 mg for both commercial preparations), it is reasonable to predict that ~17% of patients (24 of 144 patients, Huirne *et al*., 2005) will hyper-respond to that dose by a sharp drop in LH. Hypothetically, it is these patients who may benefit from added LH.

In a natural cycle, E_2 biosynthesis obeys a pre-set tide to coincide with follicular growth and ovulation (Knobil, 1974). Theca cell-derived, LH-dependent, aromatizable androgens (mainly androstenedione) are used to produce E_2 by FSH-induced granulosa cell aromatase activity. The extent of aromatase activity is limited by the amount of precursor available, which in turn depends on LH levels. In a natural cycle, LH levels are more or less constant during the follicular phase (Abraham *et al*., 1972), allowing for a sufficient supply of androgens, and for continuous rise in E_2 levels, determined by the growing number of granulosa cells in the dominant follicle and resultant increase in aromatase activity. We hypothesize that a drop in LH causes a sudden drop in precursor availability, whereas the complex system that holds a delicate balance cannot adjust to abrupt changes. The result is insufficient E_2 production by the growing follicles, manifested in a drop in circulating E_2 levels.

Physiology teaches us that LH levels during the follicular phase remain constant (Abraham *et al*., 1972). In agonist-based, pituitary down-regulation ovarian stimulation, LH levels are low, but with minimal fluctuations. Since, in a long protocol, it typically takes $\sim$2 weeks from the start of agonist treatment to ovarian stimulation, E_2 production mechanism has $\sim$12 days to respond to given LH levels. In these patients, unless LH is completely eliminated, we see a steady rise in E_2 levels during stimulation, depending on the FSH supply to the system from our FSH-containing medications. Theoretically, LH levels themselves are of less importance, as long as fluctuations are minimal. In contrast, in antagonist-based cycles following a mild decrease in LH level during the first 5 days of stimulation, a sudden antagonist-mediated LH drop leads to depleted E_2 biosynthesis (The Ganirelix Dose-Finding Study Group, 1998). We hypothesize that the drop in LH level is clinically significant, not the absolute level itself. Indeed, it was clearly demonstrated that the dynamics of LH and progesterone play a critical role in implantation when GnRH antagonist is employed. In a study of 144 women undergoing IVF, stimulated with recombinant FSH from cycle Day 2 and co-treated with various doses of GnRH antagonist, blood samples were taken three times a day for LH. Using area under the curve, adjusted for the baseline LH level before the antagonist was started, no pregnancies occurred when the LH and progesterone changed excessively during GnRH antagonist administration due to insufficient or too high doses of the GnRH antagonist (Huirne *et al*., 2005). Is there is a lesson to be learnt from this regarding individual dosing of the antagonist to achieve better pregnancy rates?

Progesterone

Progesterone is responsible for producing and sustaining a receptive endometrium during the luteal phase. If pregnancy is not achieved, progesterone (and E_2) production by the corpus luteum is shut-off and menstrual bleeding ensues. In a natural cycle, progesterone levels increase to a plateau 3–4 days after ovulation, remain constant for $\sim$6–7 days and drop to baseline. Commonly, spotting occurs during the latter part of the luteal support, as the endometrium loses its integrity in the face of decreasing progesterone levels. If pregnancy is achieved, placental-derived HCG maintains corpus luteum function and high progesterone levels.

A programmed thaw cycle in ART makes use of these events. E_2 is given to simulate the follicular phase, progesterone is added and embryo transfer is timed accordingly. Both E_2 and progesterone are continued in a constant dose to supply constant blood levels. The endometrium becomes receptive in these pregnancy-like conditions. Bleeding rarely occurs if patient compliance is good. If pregnancy is not achieved, all medications are stopped, and bleeding ensues.

In a 'fresh-embryo' ART cycle, following ovarian stimulation during the follicular phase, progesterone is commonly given as luteal support in the same doses as used in programmed thaw cycles. Surprisingly, many patients tend to bleed if pregnancy is not achieved, in spite of progesterone luteal support. Is it because of a drop in E_2 (Farhi *et al*., 2000)? Probably not, since receptive endometrium was shown to depend on progesterone only (Ghosh *et al*., 1994). What, than, could be the reason for this common phenomenon? Progesterone levels in mid-luteal phase following ovarian stimulation are very high, reflecting peak biosynthesis by numerous corpora lutea. In mid-luteal phase, the exogenously added progesterone is responsible for $\sim 10-20\%$ of total progesterone serum level; the rest is of ovarian origin. If pregnancy is not achieved, blood progesterone level quickly diminishes to a baseline supplied by the added progesterone. Although this baseline level is high enough to sustain the endometrium in a thaw cycle, we hypothesize that the endometrium 'senses' a sharp decline in progesterone resulting in diminished tissue integrity and bleeding. In other words, the change in progesterone (a sharp drop) and not the level itself is the factor that determines endometrial integrity.

Estradiol

Ovarian hyperstimulation syndrome (OHSS) is a major complication of ovarian stimulation. Accurate prediction of OHSS is still a challenge. A high E_2 level on the day of the HCG ovulation trigger is considered a major risk factor of OHSS (Papanikolaou *et al*., 2006). It is also prudent to consider the dynamics of E_2 levels until ovulation trigger day. We hypothesize that a sharp rise during a short period of time increases the probability of OHSS. Therefore, we believe, when assessing OHSS risk, not only ovulation trigger day E_2 levels must be considered but also the rate of increase during ovarian stimulation.

Human Chorionic Gonadotrophin

Hyperemesis gravidarum (HG) occurs when HCG appears in the blood. Since women with multiple pregnancy or molar pregnancy are at higher risk of developing severe HG (Fell *et al*., 2006), we hypothesize that the severity of symptoms depends not on HCG level per se but on its rise. Patients with missed abortions rarely experience HG. In fact, first trimester amelioration in HG should raise the possibility of spontaneous abortion (Furneaux *et al*., 2001).

In summary

Change is the key in the female reproductive system. Acceptance of this theory would imply that hormone measurements must be assessed in the context of time and change. We believe that changes in hormone concentrations carry significant biological messages, much more than a given level at a given time point. Understanding and proving this theory could give rise to better approaches to treatment, and risk assessment. For example, dose of GnRH antagonist would be individually adjusted according to the size of induced change in LH concentration; LH supplementation could be considered for those who prove to be over sensitive to GnRH antagonist administration with a large drop in LH. Further, more attention could be paid to androgen concentrations following down-regulation in ART. Studies are also suggested regarding the effect of change, rather than absolute levels, of progesterone on endometrial integrity. More attention should be given to the dynamics of E_2 production during ART cycles rather than absolute levels on the day of HCG for avoidance of OHSS.

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