Why frozen embryo transfer results are lower with vaginal progesterone? Did we miss something?

Priming endometrial receptivity for embryo transfers has seen a rebound in interest over the past decade. Programmed cycles in which women receive exogenous estradiol (E2) and progesterone were inherited from the early days of donor-egg assisted reproductive technology. Indeed, the high success rates of donor-egg assisted reproductive technology from inception led to use the same approach for frozen embryo transfers (FETs). However, a flurry of new alternative options has been proposed recently, and new questions have been raised. In parallel with the increase in the number of FETs performed today, many of our deeply held principles in FET have been thrown into doubt. Let us step into the core of this debate and see where we stand.

In the early days, progesterone was primarily administered by intramuscular (IM) injections; however, these were cumbersome and painful. Hence, alternatives to IM injections of progesterone have been explored. Oral progesterone is not effective because it is highly metabolized during the liver pass. Transdermal progesterone is not an option either because of the large amounts needed. Indeed, the daily production of progesterone by the corpus luteum (25 mg/24 h) is two orders of magnitude larger than that of E2 (0.05–0.5 mg/24 h), making the size of transdermal delivery systems impractical.

Vaginal administration of progesterone appeared to be the only remaining alternative for avoiding painful IM injections. Early work with vaginal progesterone provided positive returns and clinical enthusiasm. Unanticipated at first, the efficacy of vaginally administered progesterone was linked to unexpectedly high uterine tissue concentrations (1). That progesterone administered vaginally could result in such high endometrial levels was not foreseen and frankly surprising.

Intrigued by the high endometrial tissue concentration of progesterone after vaginal application, several investigators began to examine the mechanism responsible for this phenomenon. One primary question was whether a direct vagina-to-uterus transport could explain the paradox between high endometrial concentrations of progesterone and vaginal administration.

To address this issue, Bulletti et al. (2) used an ex vivo model system with hysterectomized uterus. Using an open system (no recirculation) and application of radiolabeled progesterone to the cervical cuff, labeled progesterone was progressively recovered within the whole uterus, reaching the fundus after 6 hours of perfusion. This direct vagina-to-uterus transport, or *first uterine pass effect*, is associated with a countercurrent exchange system. Such a system results from a direct vein-to-artery diffusion followed by retrograde transport into the uterus and a special arrangement of the upper vaginal vascular anatomy with close contact between the venous and arterial systems. Ultimately, on the basis these studies and others, the concept of a *first uterine pass effect* in case of vaginal administration of progesterone became recognized. Logically, this led progressively to abandon interest in the circulating levels of progesterone after vaginal administration because of the high endometrial concentration.

By and large, most European clinicians began primarily to use vaginal progesterone—for example, capsules and gel—for priming endometrial receptivity for FETs. Conversely, most US practitioners remained faithful to the old-fashion IM injections of progesterone.

With the first uterine pass effect being widely recognized and accepted, administration of vaginal progesterone was used for many years. In 2018, however, a randomized controlled trial was published that raised uncertainty about the efficacy of vaginal progesterone for hormonal priming in FETs (3). This 3-arm randomized controlled trial included one group that only received vaginal progesterone after E2 priming, one that used IM progesterone (50 mg/d), and one that used a combination of vaginal progesterone and IM progesterone every third day. A planned interim analysis of the trial concluded that the pregnancy rates were lower and early pregnancy losses were higher in the group receiving vaginal progesterone only (3). This led to early termination of the vaginal progesterone group although the study continued with the two remaining groups.

Subsequently, several studies suggested that the serum levels of progesterone actually played a role in the efficacy of vaginal progesterone. Labarta et al. (4) reported that women whose serum progesterone levels were <10 ng/mL on the day of transfer had lower results than those whose progesterone levels were higher. Conversely, results were normalized if these women were supplemented using newly available subcutaneous progesterone injections. These data were ultimately confirmed by a plethora of similar investigations—and a meta-analysis—which all pointed to a correlation between serum progesterone and FET outcome.

How is this possible? Vaginal progesterone offers a selective delivery to the uterus—through a *first uterine pass effect*. Yet, recent publications suggest that the serum progesterone levels matter and play a role in FET outcome. How would this be? Is it not the endometrial concentration of progesterone that controls receptivity? If so, why are we talking about circulating levels of progesterone?

The numerous publications that pointed at a critical role of circulating levels of progesterone forced us, however, to reconsider our views. What if we have omitted a player in the process endometrial priming when solely focusing on the endometrial concentration of progesterone? What if staring at the high endometrial concentration of progesterone has led us to turn a blind eye on some nonpelvic effects of progesterone? What if these nonpelvic effects of progesterone could be instrumental in the development of pregnancy?

One mystery of developing pregnancies is the nonrejection of the conceptus by the maternal immune system. Indeed, the conceptus should be seen as an intruder. However, pregnancy is associated with an intriguing state of immunotolerance, which we may have ignored with our focus on endometrial effects of progesterone. Yet, progesterone also

exerts potent immunomodulatory functions throughout pregnancy. Notably, progesterone promotes the differentiation of regulatory T cells both systemically and at the maternal-fetal interface (5). Progesterone also decreases the cytotoxic activity of natural killer cells and induces an anti-inflammatory phenotype, which supports immunotolerance (5). Together, these progesterone-driven immunologic changes foster a homeostatic state that ensures a successful pregnancy (5). Importantly for our discussion here, these effects of progesterone on the immune system are, for the most part, mediated outside of the pelvis. Hence, these effects are serum level dependent, not endometrial tissue concentration related.

So, would it not be time for us to pause and ask ourselves whether our enthusiasm for the endometrial effects of progesterone has not blurred our vision? What if focusing on the endometrium had led us to ignore the nonpelvic effects of progesterone and notably its role on initiating immunotolerance? Did we not remain steadfastly focused on what had profoundly intrigued us, the direct transport of vaginal progesterone to the endometrium, to the point of ignoring and forgetting the rest? Did we not miss something? It certainly seems so. Thus, it is time that we reset the clock and admit our omissions, even if they stemmed from a laudable interest of a scientific puzzle, the *first uterine pass effect*. In programmed preparation for FET, serum progesterone levels matter. We should use either injectable progesterone or, possibly, a mixture of vaginal and injectable progesterone and be cognizant of the immunologic role played by progesterone.

CRediT Authorship Contribution Statement

Dominique de Ziegler: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Sean Soktean:** Writing – original draft, Writing – review & editing. **Paul Pirtea:** Writing – original draft, Writing – review & editing.

Declaration of Interests

D.d.Z. has nothing to disclose. S.S. has nothing to disclose. P.P. has nothing to disclose.

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