Editorial



# New insights in addressing endometrial dysfunction: the potential role of growth hormone

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*Provenance and Peer Review:* This article was commissioned by the Editorial Office, *Annals of Translational Medicine*. The article did not undergo external peer review.

*Comment on:* Feng Q, Gao B, Huang H, *et al.* Growth hormone promotes human endometrial glandular cells proliferation and motion through the GHR-STAT3/5 pathway. Ann Transl Med 2020;8:53.

Submitted Mar 24, 2020. Accepted for publication Apr 10, 2020.
doi: 10.21037/atm.2020.04.53
**View this article at:** http://dx.doi.org/10.21037/atm.2020.04.53

Feng and colleagues recently published, in the journal "*Annals of Translational Medicine*" a basic research study aiming to investigate the possible effect of growth hormone (GH) on the physiology of cultured human endometrial glandular cells (hEGSc), targeting the growth hormone receptor (GHR)-STAT3/5 pathway (1). This is a highly interesting study that raises important issues to be discussed, and merits further analysis and understanding as it provides significant evidence. Feng and colleagues focus on the hypothesis that GH could be an essential molecule for endometrial rejuvenation when endometrial dysfunction is identified, especially on the grounds of intrauterine adhesions (IUAs) presence.

The rationale fueling design and performance of this study was the significant prevalence of endometrial dysfunction in infertile couples presenting with implantation failure, coupled by the absence of a universally accepted effective treatment protocol (1). IUAs sometimes are symptomatic and cause abnormal menstruation phenomena, such as amenorrhea or hypermenorrhea (2). Authors note, that following on the causative relationship with secondary infertility, endometrial dysfunction-including IUAs—may in fact contribute to significantly jeopardize even the quality of life of patients. Novel treatments towards addressing multifaceted health issues such as IUAs are of heightened value especially in light of the lack of foolproof current treatments. Nonetheless, they merit thorough investigation. Studies like the study of Feng *et al*., are to be highly commended for recruiting basic research in the service of providing significant evidence on such novel treatments. Some recently emerging novel treatments towards improving endometrial function are employment of GH or platelet-rich plasma (PRP) (3). GH use for endometrial rejuvenation is emerging as particularly promising albeit lacking "clinical routine practice" status. Hence this study, providing data on the use and mode of action of GH may be viewed as ultimately reporting "back to the practitioner". This may add further value to the study's appeal. In the field of Reproductive Medicine, we may have become accustomed to novel treatments employed-all too soon-towards overcoming infertility issues. The phenomenon of application of novel therapeutic methods in clinical practice prior to ascertaining efficiency is aptly raising several concerns within the scientific community. The paradigm of Feng and colleagues on employing appropriate research methodology in order to understand the mechanism behind something that clinical observation may find effective, should be applauded and followed prior to introducing novel treatments in clinical practice.

Considering endometrial dysfunction, it is well established that IUAs constitute the most frequent long-term complication of dilation and curettage and usually

occur as a consequence of endometrial and myometrial layer injury (2,4). Dilation and curettage, inevitably represent an integral part of the daily clinical routine, and have a wide range of applications in both diagnostic and therapeutic procedures, such as management of spontaneous abortion (4). Intrauterine synechiae aggravate when continuous invasive procedures in the endometrial cavity or the use of sharp curettage are employed (2).

Several interventional treatments for treating IUAs have been suggested albeit results remain controversial. Initially, the therapeutic trend was cervical probing and curettage, however, the plethora of side effects and high rates of adhesions recurrence led the methods' rejection. Hence, hysteroscopy has been the gold standard treatment enabling simultaneous diagnosis and co-treatment of IUAs (5). Over time, the technique has improved with significant results in restoring fertility (2). Nonetheless, disadvantageous aspects of hysteroscopy have also emerged. More specifically, reported complications include perforation of the uterus ranging from 2% to 5%, severe hemorrhage in 6% to 27% of the population, and cervical incompetence following continuous dilations (2,6). Ultimately, female fertility is understandably compromised, even though surgical symphysiolysis may have aimed at the opposite outcome.

In the context of adjuvant treatment, scientific interest has been focused on the use of several different approaches, including insertion of intrauterine devices and balloons, estradiol administration and other hormonal treatments, barriers gels and human amniotic membrane drafting, so as for the formation of IUAs to be prevented (2,7). Published data indicate that the quality of evidence, in regard to the effectiveness as well as the safety of these approaches, is poor. Thus, no safe conclusions can be extracted (7). Despite the initial promise, it is now apparent that there are no robust data suggesting the use of any of the above-mentioned adjuvant therapies following hysteroscopic lysis of IUAs, in order to prevent IUA formation or in order to improve the reproductive dynamic for these patients (7). At the same time, studies highlight the risk of a uterine infection-such as chronic endometritis-when opting for adjuvant treatments for prevention of IUA formation following hysteroscopic lysis (8,9).

Taking into account the aforementioned, as well as acknowledging that GH is one of the master regulators of growth and development, Feng *et al.*, posed the question of whether GH administration could enhance hEGSc performance in *in vivo* culture system (1). Authors proceeded to hEGSc isolation from endometrial biopsies and cultured these cells in 10% FBS DMEM/F12 (1). It would be of added value had information regarding the population from which endometrial biopsies were received been provided. Provision of data regarding the patients' age, reproductive history, possible existing endometrial pathologies, along with a report on the microbiological environment of these samples, would certainly enrich the study. When experiments performed on home-made cell lines originating from tissue biopsies, it is imperative to ensure that these cells are of high quality and represent a healthy and fully functional endometrium.

Following isolation, authors proceeded to several experiments, performing a robust all-inclusive analysis with respect to both gene expression as well as the protein production level. In order to investigate the potential effectiveness of GH in hEGSc performance, Feng's foolproof study design dictated exposure of these cells to increasing concentrations of GH or co-exposure to GH and AG490. The latter being selected on the grounds of its specific and potent inhibition of the Janus kinase 2 protein (JAK2) (1). It is well demonstrated that one of the classical intracellular signaling pathways induced by GH is the JAK/STAT pathway (10). Thus, the authors' choice to use the JAK inhibitor AG490 was critical in order to prove that GH acts in hEGSc via the JAK/STAT pathway, as this was one of their primary goals in testing their hypothesis (1).

Results presented in the study of Feng *et al.*, are of significant merit. Employing the classical MTT and EdU proliferation assays, authors proved that GH significantly promoted hEGSc viability and proliferation. Furthermore, GH significantly increased the proportion of hEGSc in the G2 and S phase of cell cycle, and reduced the number of those in G1 phase. This was demonstrated employing flow cytometry analysis, verifying that GH is able to promote hEGSc proliferation which is required for endometrial rejuvenation. This commentary highlights the most impressive part of this study, being the hEGSc migration capability assay. Authors designed an *ex vivo* system, mimicking injury conditions in the endometrial cavity, in order to assess GH's ability to promote migration of hEGSc towards restoration of the "injured" cell layer. These conditions are observed following lysis of the adhesions. Results indicated that GH could effectively promote hEGSc migration demonstrating endometrial tissue restoration abilities. The aforementioned GH's effects were observed when hEGSc were exposed to high concentrations of GH, namely 100 and 200 ng/mL, and all of the above-mentioned GH's actions were significantly suppressed when the hEGSc

 

co-exposed with the JAK inhibitor AG490. Collectively, these results demonstrate that GH may promote hEGSc proliferation, may activate hEGSc cell cycle, as well as enhance hEGSc migration capability, directly via the JAK/STAT pathway (1).

In regard to gene expression and the protein production levels, results point-out that GH supplementation in culture media notably increased GHR expression, as demonstrated via immunocytochemistry using specific anti-GHR antibodies and significantly activated STAT 3 mRNA expression, as indicated by RT-PCR analysis. Authors failed to provide evidence indicating GH's effect on STAT 3 protein levels. Moreover, GH supplementation had no effect neither to STAT 5 or mRNA and protein levels. However, GH induced phosphorylation of both STAT 3 and 5 proteins, being the active protein forms. As anticipated, when the hEGSc became co-exposed to GH and to the JAK inhibitor AG490, an extensive reduction was observed in regard to mRNA levels of both STAT 3 and STAT 5, as well as, in regard to STAT 3 and STAT 5 and their phosphorylated isomorphisms (1).

Despite these encouraging findings, and as authors aptly point out, additional studies are required in order to unveil the intracellular mechanisms involved in GH's intracellular signaling in hEGSc. Such data will contribute towards enriching our knowledge in regard to GH's potential therapeutic value. In the study analyzed herein, authors chose to investigate the classical intracellular signaling promoted by GH, which is the JAK/STAT pathway, investigating only the STAT 3 and 5 molecules. This commentary submits the thesis that it would be of considerable interest to similarly investigate several other proteins playing crucial roles in this pathway, including the JAK proteins, phosphotyrosine phosphatases (PTPs) and the suppressors of cytokine signaling (SOCS), which down regulate the JAK/STAT pathway (10-12). In this context, it would be a noteworthy advantage to investigate the GH-JAK2-depented phosphorylation of insulin receptor substrate (IRS) pathway, which activates the major PI-3 kinase/Akt pathway playing a crucial role on cytoskeleton modification (10,13). Moreover, it is well showcased that GH could activate a variety of intracellular signaling cascades-other than JAK related pathways-including the c-Src family of protein kinase, affecting the metabolic function of cells (10,14). Considering the above-mentioned, transcriptomic and proteomic analysis in hEGSc following GH supplementation would add another level of value to the investigation. Future studies are pivotal to examine conclusively GH induced intracellular processes, in various cell types identified in the endometrium. Such research would provide the scientific community with in-depth knowledge, prior to employing GH as a treatment to overcome IUAs' detrimental impact on endometrial function compromising fertility status.

Data presented by the study of Feng *et al*. (1), are strongly supported by the findings of Cui *et al*., also recently published (15). In this study, authors research the potential effectiveness of GH aiming to improve reproductive outcome in women presenting with thin endometrium. Results indicate that patients receiving GH administration via subcutaneous injections of 5 IU of GH daily, presented with increased endometrium thickness on day 3 of the menstrual cycles, coupled by higher implantation and clinical pregnancy rates compared to the control group constituting of women receiving only routine exogenous estrogen administration (15). In the same study, authors investigate the potential role of GH supplementation in the culture media of human endometrial carcinoma cell line RL95-2 (15). Respective results are in the same line with the results presented by Feng *et al*., indicating that GH promotes RL95-2 cell proliferation and viability, while increasing the proportion of cells RL95-2 existing on the G2 and S phases of cell cycle. In addition, GH induced expression of significant proteins, adhesion molecules and growth factors that are related to normal endometrial function. These include vascular endothelial growth factor (VEGF), integrin beta-3 (ItgB3) and insulin-like growth factor-I (IGF-1). Especially in regard to IGF-I, data demonstrate that several of GH's effects on the endometrium are enabled via paracrine and autocrine phenomena mediated by the GH-IGF axis (15,16). Had this noteworthy observation, been investigated in the study of Feng *et al*., it would have heightened its value.

In conclusion, employment of GH for treatment of IUAs has not yet been in-depth researched and fully evaluated. GH has hitherto been examined as an adjuvant therapy for a number of infertility cases including patients with hypogonadotropic hypogonadism or panhypopituitarism, poor responders, women with thin endometrium or recurrent implantation failure (17,18). It appears timely and essential to proceed with a clinical evaluation of GH on patients with IUA. However future research should abide by the fundamental values of evidence-based medicine. The first step is to proceed with additional studies of high quality of evidence, such as the one contributed by Feng and colleagues that successfully set the tone for further research.

 

Investigation should focus on fully unraveling the potential benefits of GH administration on a cellular level addressing qualitative as well as quantitative matters. Secondly, evaluation in animal models should be a prerequisite, in order to concur on the optimal required dose, as the effect may be modified when proceeding from cell lines to living organisms. In the future, perhaps the technology of organ-on-a-chip may replace the necessity for extensive animal studies and enable safety of drugs administration to humans, as this model will allow successful prediction of respective toxicity (19). Phase I and II clinical trials should follow, recruiting small numbers of participants, in order to ascertain safety of the procedure as well as the optimal dosage in humans. Possible co-administration of GH with other hormones, such as estrogens, should be attempted. Treatment efficacy as well as possible side-effects should be primary outcomes and all comparisons should be performed against the gold standard for diagnosis and treatment. The impact of GH administration for endometrial rejuvenation regarding reproductive hormone levels, clinical pregnancy, live-birth and miscarriage rates should be accounted for. Additionally, multi-center studies-when properly designed-may ascertain less bias and are of added value prior to introducing GH treatment in clinical practice. Ultimately, it is only large randomized controlled trials reporting on a variety of outcome measures that will convey safety, and lift the "experimental' classification status replacing it with that of "clinical routine practice".

## Acknowledgments

*Funding:* None.

## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm.2020.04.53). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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**Cite this article as:** Grigoriadis S, Maziotis E, Vlahos N, Simopoulou M. New insights in addressing endometrial dysfunction: the potential role of growth hormone. Ann Transl Med 2020;8(14):841. doi: 10.21037/atm.2020.04.53

 