Endometrial thickness on the day of embryo transfer is a poor predictor of IVF treatment outcome

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Submitted on November 14, 2017; resubmitted on December 12, 2017; editorial decision on December 29, 2017; accepted on December 30, 2017

STUDY QUESTION: What is the independent contribution of endometrial thickness (EMT) on day of embryo transfer to achieving an ongoing pregnancy and live birth after IVF treatment?

SUMMARY ANSWER: EMT is a poor predictor of IVF success and has only little independent prognostic value.

WHAT IS KNOWN ALREADY: In a number of previous studies, pregnancy rates have been found to be lower in patients with thin endometrium.

STUDY DESIGN, SIZE, DURATION: This is a retrospective analysis of data from two large, randomized phase III studies (conducted in Europe and the USA) comparing s.c. progesterone with vaginal progesterone for luteal phase support. The studies were very similar in design, patient population and outcome, and the study data were combined and analysed on an individual patient level.

PARTICIPANTS/MATERIALS, SETTING, METHOD: Subjects were infertile patients with an indication for IVF/ICSI, aged between 18 and 42 years, BMI <30 kg/m2, <3 prior ART cycles and ≥3 oocytes after controlled ovarian stimulation with GnRH-agonist or GnRH-antagonist. EMT was assessed on day of embryo transfer (n = 1401). The association of EMT and ongoing pregnancy rate was determined by comparison of outcomes by quartiles of EMT. The predictive capacity of EMT for ongoing pregnancy achievement was assessed at each millimeter cut-off. Finally, a regression model was built to determine the contribution of EMT among other confounders, such as age and oocyte numbers, on the likelihood of ongoing pregnancy and live birth.

MAIN RESULTS AND THE ROLE OF CHANCE: In univariate analysis, ongoing pregnancy rates correlate to EMT. In patients above a cut-off of ≥9 mm EMT, the chance of pregnancy was higher as compared to patients with an EMT of 3–8 mm (odds ratio (OR) = 1.69, 95% CI: 1.23–2.35, P = 0.001; sensitivity 88.89%, specificity 17.52%, positive predictive value 39.02%, negative predictive value 72.64% and likelihood ratio 1.08). In multivariate regression analysis, after controlling for trial, female age and oocyte numbers, EMT was a statistically significant predictor of live birth (OR = 1.05, 95% CI: 1.00–1.10; P = 0.0351). If EMT indeed is an independent factor affecting outcome, this finding implies that at a baseline live birth rate of 20% an increase of 2 mm in EMT should result in an increase of the live birth rate of ~1.6%.

LIMITATIONS REASONS FOR CAUTION: The independent contribution of EMT to live birth likelihood is small and may result from (undetermined) confounding. The EMT on day of embryo transfer is usually higher as compared to the EMT on day of triggering final oocyte maturation when it is conventionally assessed during routine cycle monitoring. Furthermore, endometrial lining pattern and/or subendometrial Doppler flow have not been assessed and, accordingly, the conclusions of this work are limited to only the thickness of the endometrium.

WIDER IMPLICATIONS OF THE FINDINGS: EMT can be ignored during cycle monitoring of the majority of IVF patients and only the extremes of EMT deserve further diagnostic work-up.
Introduction

Endometrial thickness (EMT), measured in the sagittal plane by transvaginal ultrasound and expressed in millimetres, is routinely assessed during infertility work-up and treatment. A number of observational studies have assessed the association of EMT with the chance of achieving a pregnancy or live birth after IVF, but with conflicting results (De Geyter et al., 2000; Schild et al., 2001; Jarvela et al., 2005; Alcazar, 2006; Ng et al., 2006; Wang et al., 2010; Bu and Sun, 2013). To this day, clinicians and patients are worried about the occurrence of a ‘thin endometrium’ and some clinicians advocate cycle cancellation, freezing of all embryos for transfer in a frozen-thawed cycle under natural conditions, extended use of estrogens in an artificial frozen-thawed cycle (Chen et al., 2006) or the use of various adjuncts such as granulocyte colony stimulating factor (Xu et al., 2015; Check et al., 2016), hCG or prostaglandins (Nakagawa et al., 2014) to increase EMT.

A recent systematic review and meta-analysis (Kasius et al., 2014) concluded that the EMT, assessed on the day of triggering final oocyte maturation, has a limited capacity to identify women who have a low chance to conceive after IVF, while acknowledging that below a cut-off of 7 mm a lower chance of pregnancy can be observed in univariate analyses. However, it is unclear whether the EMT is more an epiphenomenon of potentially multiple predictive factors for IVF success, such as ovarian response, age and patient history, rather than an independent contributor to the chance of achieving a pregnancy. Accordingly, the authors of the systematic review (Kasius et al., 2014) called for further research to investigate the real independent significance of EMT in IVF.

Having available the complete dataset of two recently conducted, large, multicentric, multinational, phase III, IVF trials allows the study of which patient characteristics are associated with EMT. Furthermore, the association of EMT with treatment outcome in univariate and multivariate analyses can be assessed. Thereby, a best estimate of the independent contribution of EMT to IVF success can be determined.

Materials and Methods

The present study is a retrospective analysis of data from two large phase III studies (07EU/Prg06 and 07USA/Prg05) conducted in Europe and the USA, respectively (Baker et al., 2014; Lockwood et al., 2014). Both studies were prospective, multicentre, open, randomized, parallel, two-armed trials. The studies were conducted to establish non-inferiority of ongoing pregnancy rate in patients undergoing IVF or ICSI and receiving luteal phase support with daily s.c. injections of 25 mg progesterone as compared to vaginally administered progesterone gel 90 mg (Lockwood et al., 2014) or progesterone tablets 100 mg bid (Baker et al., 2014). Details of the trials have previously been reported (Baker et al., 2014; Lockwood et al., 2014). In brief, patients were randomized on the day of oocyte retrieval and started luteal phase support on the same day. No statistical or clinical significant differences were detected between s.c. and vaginal progesterone for luteal phase support, both when examining the individual trials, as well as when combining the data from the two studies at an individual patient level (Doblinger et al., 2016). Both studies were performed in accordance with the Good Clinical Practice guidelines as defined by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and international regulations, such as the Helsinki Declaration.

Patient and study characteristics

A total of 1483 patients were randomized in the two studies (683 and 800 patients, respectively). In brief, the studies had highly similar inclusion
criteria: female infertile patients with an indication for IVF or ICSI, age between 18 and 42 years, BMI <30 kg/m², <3 prior ART cycles (IVF, ICSI and related procedures), baseline (cycle Day 2 or 3) FSH < 15 IU/L and estradiol-17β (E₂) <80 pg/mL, a normal uterine cavity as per recent hysterosalpingogram, sonohysterogram or hysteroscopic exam (i.e. no polyp or protruding sub-mucosal fibroid), and at least three retrieved oocytes after controlled ovarian stimulation. Relevant common exclusion criteria were: cavity-distorting intramural fibroids, stage III or IV endometriosis, hydrosalpinx, history of previous poor response, recurrent miscarriage, adrenal or thyroid disease, and thromboembolic disease or disorder.

Eligible patients were allowed any kind of LH suppression (agonist or antagonist with or without oral contraceptive pill pre-treatment) during controlled ovarian stimulation and any gonadotrophin stimulation regimen (recombinant or urinary FSH, HMG or a combination at doses individually determined by the treating physicians). Oocyte maturation was performed with 5000–10 000 IU of hCG (except for one case in trial 07EU/Prg06 in which GnRH-agonist had been used). Transfer could occur at either the cleavage or blastocyst stage. The number of embryos transferred was at the discretion of the investigator and the patient. EMT was assessed on the day of embryo transfer in the mid-sagittal plane. No protocol specifications existed on a minimally necessary EMT.

**Outcome parameters**

An ongoing pregnancy was defined as a viable pregnancy 10 weeks after oocyte retrieval. A live birth was defined as the birth of at least one viable infant.

**Statistical analyses**

Statistical heterogeneity between the trials was tested by applying an I² statistic to the aggregated risk difference of ongoing pregnancy to determine the percentage of variation across trials caused by heterogeneity as opposed to sampling error. The I² statistic was 0% (P = 0.99), suggesting no significant heterogeneity between trials and, accordingly, the data from the two trials were combined and used on an individual patient level.

The distribution of EMT was assessed. EMT as well as other demographic and treatment parameters were then compared between patients achieving pregnancy or not, as previously described (Doblinger et al., 2016). The association of demographic variables and EMT was examined to identify predictors of extremes of EMT. To determine the univariate association between EMT and ongoing pregnancy rate, a comparison of outcomes by quantiles of EMT was performed. The association of EMT and ongoing pregnancy rate was tested by Mantel–Haenszel chi-square and a test for a linear trend was performed by calculating Spearman’s and Pearson’s correlation coefficients.

Next, the predictive capacity of EMT was tested for each millimeter cut-off. The ongoing pregnancy rate was compared below and above each millimeter threshold to determine the optimal cut-off of EMT. An odds ratio (OR) with 95% CI was calculated for the chance of pregnancy when the EMT was above the cut-off together with P value, sensitivity, specificity, negative predictive value, positive predictive value and likelihood ratio. Furthermore, a receiver operating characteristic curve (ROC) analysis was carried out.

Results are presented descriptively, as n, mean, and SD for continuous variables or frequencies, and percentages for categorical variables, as appropriate. For continuous variables, differences between groups were analysed by ANOVA or the Mann–Whitney U-test; for ordered categorical variables, the groups were compared using the Cochran–Mantel–Haenszel test with modified ridit scores, while for non-ordered categorical variables, the chi-square test or the Fisher’s exact test was used.

For the multivariate analysis, a logistic regression model was fitted with trial included as a fixed effect dummy that allowed the log odds to vary across the trials. At first, this model was used to assess separately the relationship between each parameter—women’s age and BMI, previous pregnancies leading to a live birth, duration (months) and cause of infertility (female factor, male factor, unexplained and combined), baseline FSH level, EMT, number of oocytes retrieved, type of treatment (IVF or ICSI), number of embryos transferred and transfer difficulty—and the outcome. With the EMT forced-in, all these covariates were included in a stepwise logistic regression modelling procedure, to determine multiple covariates that, together, had a significant effect on outcome. Then, effects for predictors of ongoing pregnancy and live birth selected from this model were inserted in a multivariate logistic model while holding the trial effect fixed, as previously described. The OR and 95% CIs were calculated taking into account all patients from the intention-to-treat population who underwent embryo transfer (per-protocol analysis).

All P values were rounded off to three decimals. Statistical significance was defined as a P value <0.05. All statistical analyses were performed using the software package SAS® system version 9.4 (SAS Institute Inc., Cary, NC, USA).

**Results**

The combined sample size of the randomized patient population was 1483 patients, the number of per-protocol patients (patients undergoing embryo transfer) was 1435, and EMT measurements were available for 1401 patients. No patient was cancelled between randomization and embryo transfer because of insufficient EMT. Reasons for drop-out between randomization and embryo transfer were failed fertilization or embryonic arrest (n = 34), risk of ovarian hyperstimulation syndrome (OHSS) (n = 11), OHSS (n = 1), no sperm (n = 1) and allergic reaction (n = 1).

Figure 1 shows the distribution of EMT on day of embryo transfer. EMT in the majority of patients (85.65%) was between 8 and 15 mm.

**Predictive factors associated with EMT**

As depicted in Table I, mean EMT tended to be less with higher age and usage of GnRH-antagonists, but the absolute differences are small and not statistically significant. Only oocyte number is significantly related to EMT, e.g. the more oocytes collected, the higher the EMT.

**Ongoing pregnancy rates by quantiles of EMT**

Figure 2 depicts ongoing pregnancy rates with 95% CIs by quantiles of EMT. The ongoing pregnancy rate in patients with EMT ≤8 mm was 29.1% (95% CI: 21.60–37.8%). An increase of the ongoing pregnancy rate with increasing EMT was observed (Mantel–Haenszel chi-square P = 0.042). Spearman’s and Pearson’s correlation coefficients indicated a positive, yet weak linear trend (r = 0.0537 and r = 0.0543, respectively).

**Optimal cut-off of EMT**

Figure 3 depicts ORs with 95% CIs for ongoing pregnancy achievement for different cut-offs of EMT. It can be seen that the point estimates of the OR are consistently in favour of higher EMT. However, statistical significance is only found for the cut-off ≥ 9 mm EMT. Ongoing pregnancy likelihood was significantly higher in patients with an EMT ≥ 9 mm as compared to patients with an EMT 3–8 mm (OR = 1.69, 95% CI: 1.23–2.35, P = 0.001; sensitivity 88.89%, specificity 17.52%, positive predictive value 39.02%, negative predictive value 72.64% and likelihood ratio 1.08). Supplementary Figure S1 depicts the ROC curve,
Figure 1  Distribution of patients by endometrial thickness on the day of embryo transfer. Mean (±SD) and median endometrial thickness were 11.08 (±2.40) and 10.90, respectively.

Table I Predictive factors associated with endometrial thickness.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>≤8 mm (n = 117)</th>
<th>8.1–15 mm (n = 1200)</th>
<th>&gt;15 mm (n = 84)</th>
<th>Mean EMT (mm)</th>
<th>P value (for percentage)</th>
<th>P value (for mean)</th>
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<tr>
<td>All cycles</td>
<td>1401</td>
<td>8.35</td>
<td>85.65</td>
<td>6.00</td>
<td>11.08 ± 2.40</td>
<td>&lt;0.0001</td>
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<td>Age (years)</td>
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<td>18–25</td>
<td>45</td>
<td>11.11</td>
<td>80.00</td>
<td>8.89</td>
<td>11.47 ± 2.47</td>
<td>0.107</td>
<td>0.271</td>
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<tr>
<td>26–35</td>
<td>836</td>
<td>8.73</td>
<td>84.93</td>
<td>6.34</td>
<td>11.13 ± 2.50</td>
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<tr>
<td>36–40</td>
<td>453</td>
<td>7.51</td>
<td>86.98</td>
<td>5.52</td>
<td>11.01 ± 2.25</td>
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<td></td>
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<tr>
<td>&gt;40</td>
<td>67</td>
<td>7.46</td>
<td>89.55</td>
<td>2.99</td>
<td>10.67 ± 2.06</td>
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<td>Female</td>
<td>394</td>
<td>10.66</td>
<td>84.77</td>
<td>4.57</td>
<td>10.98 ± 2.38</td>
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<td>Male</td>
<td>549</td>
<td>8.20</td>
<td>84.15</td>
<td>7.65</td>
<td>11.11 ± 2.54</td>
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<td>Combined</td>
<td>254</td>
<td>6.30</td>
<td>88.98</td>
<td>4.72</td>
<td>11.31 ± 2.23</td>
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<td>Unexplained</td>
<td>204</td>
<td>6.86</td>
<td>87.25</td>
<td>5.88</td>
<td>10.89 ± 2.25</td>
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<tr>
<td>Medication type*</td>
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<tr>
<td>GnRH-agonist</td>
<td>1066</td>
<td>7.97</td>
<td>85.08</td>
<td>6.94</td>
<td>11.15 ± 2.45</td>
<td>0.171</td>
<td>0.070</td>
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<td>GnRH-antagonist</td>
<td>321</td>
<td>9.03</td>
<td>87.85</td>
<td>3.12</td>
<td>10.88 ± 2.24</td>
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<td>Both</td>
<td>13</td>
<td>23.08</td>
<td>76.92</td>
<td>0.00</td>
<td>9.61 ± 1.56</td>
<td></td>
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<tr>
<td>No. of oocytes retrieved</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>2–3</td>
<td>48</td>
<td>10.42</td>
<td>89.58</td>
<td>0.00</td>
<td>10.49 ± 1.71</td>
<td>0.345</td>
<td>0.010</td>
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<td>4–5</td>
<td>144</td>
<td>9.03</td>
<td>86.81</td>
<td>4.17</td>
<td>10.63 ± 2.21</td>
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<tr>
<td>≥6</td>
<td>1209</td>
<td>8.19</td>
<td>85.36</td>
<td>6.45</td>
<td>11.16 ± 2.44</td>
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</table>

*For one patient this information was not available. EMT, endometrial thickness. (P-values calculated by chi-square for percentages and ANOVA for means.)
which indicates a poor performance of the EMT to predict ongoing pregnancy (area-under-the-curve: 0.53; 95% CI: 0.50–0.56).

**Multivariate analysis**
In patients achieving ongoing pregnancy, female age was lower (33.1 versus 34.3 years, \( P < 0.0001 \)), while the number of oocytes retrieved (14.5 versus 12.4, \( P < 0.0001 \)) and EMT (11.25 versus 10.98 mm, \( P = 0.04 \)) were significantly higher than in patients who did not achieve pregnancy. Embryo transfer was more often judged moderately or extremely difficult in patients not achieving pregnancy (8.2 versus 4.5%, \( P = 0.04 \)). All other potential predictors were not found to differ.

In the logistic regression analysis, EMT was statistically significantly associated with ongoing pregnancy rate (OR = 1.057, 95% CI: 1.01–1.11; \( P = 0.0167 \)), after controlling for trial, female age, transfer difficulty and oocyte numbers, where trial was considered as a fixed effect and the other variables were chosen from a stepwise logistic regression. Likewise, EMT was statistically significantly associated with the likelihood of a patient to achieve live birth (Table II).

**Discussion**
The present study is based on a broad population of patients undergoing controlled ovarian stimulation and IVF with fresh embryo transfer showing a broad range of EMT on the day of transfer. Approximately 5% of the population had an EMT of \( \leq 7 \) mm on day of embryo transfer, which is more than the 2.7% reported previously from multiple studies, in which EMT was measured on day of hCG (Kasius et al., 2014). This is noteworthy, since the increase in EMT from late follicular phase to luteal phase has been estimated as \( \sim 1 \) mm (Barker et al., 2009). This therefore implies that the phase III study population analysed herein is unlikely to suffer from selection bias towards ‘thicker endometrium’.

The increase in EMT induced by secretory transformation is potentially the reason for the differences in cut-off below which pregnancy chance is predicted to be lower as reported here (9 mm) compared to previous reports (7 mm) (Kasius et al., 2014). In our data with EMT assessed on day of embryo transfer, a cut-off of 9 mm could predict ongoing pregnancy, but the predictive performance was poor overall and also highly similar to the poor test characteristics reported by Kasius et al. (2014). A likelihood ratio of 1.08 implies only a small and clinically insignificant change in a patient’s chance to achieve an ongoing pregnancy if the endometrium is above 9 mm diameter. The present data therefore reinforce the message that EMT alone has little predictive capacity for the occurrence of pregnancy (Kasius et al., 2014) and put this conclusion on a broader basis since the EMT was measured and analysed, for the first time, in the context of larger IVF studies, on the day of embryo transfer.

Numerous previous studies have identified oocyte numbers and female age as potential confounders of EMT, for example the higher the age and the lower the oocyte number, the thinner the endometrium (Bozdag et al., 2009; Chen et al., 2010; Kuc et al., 2011). In the current analysis, a positive association of oocyte numbers and EMT...
was found in univariate analysis. To test the independent contribution of EMT to ongoing pregnancy and live birth likelihood, a meta-analysis of the two studies at the level of individual patient data was performed. In logistic regression analysis it was found that per unit increase of the EMT, the odds for live birth changes by the factor 1.05. It is important to note that this small change in the odds for live birth may still result from unknown confounding and, accordingly, this observational study must not be taken as definite proof that EMT is indeed impacting the chance of pregnancy and birth. However, the data presented here may serve to help understand what effect can possibly be expected from clinical interventions that aim at increasing the EMT, such as prolonging the E2 treatment in a frozen–thawed cycle: at a live birth rate of 20% an increase of 2 mm in EMT should result in an increase of ~1.6% in live birth rate; at a pregnancy rate of 30% an increase of 2 mm in EMT should result in an increase of ~2.4% in live birth rate. It is clear from these examples that the potentially independent contribution of EMT to a positive outcome is too small to be determined with confidence in clinical trials of reasonable size.

It is reassuring that a recently published retrospective analysis of a large number of IVF cycles arrived at a similar estimate of the association of EMT and live birth (OR=1.078; P < 0.001) after controlling for female age, the only significant predictor in a stepwise regression analysis (Yuan et al., 2016).

The protocol of the phase III studies dictated EMT measurement to be performed on day of embryo transfer, since patients were only randomized on day of oocyte retrieval if at least three oocytes had been collected. Endometrial lining pattern has not been assessed and little is known about pattern differences in early luteal phase. Also for the day of triggering, different classification systems for endometrial pattern have been used in previous studies, making a collation of data from multiple studies a difficult task (Kasius et al., 2014). At present it appears as if for the clinical utility of endometrial pattern assessment, no clear message can be derived from conflicting study results (Dietterich et al., 2002; Rashidi et al., 2005; Dechaud et al., 2008; Bozdag et al., 2009; Chen et al., 2010; Kuc et al., 2011; Singh et al., 2011; Zhao et al., 2012).

**Figure 3** Odds ratios with 95% CIs for ongoing pregnancy achievement for different cut-offs of endometrial thickness. OR = odds ratio; EMT = endometrial thickness.
Beyond the naturally occurring variation in EMT between individuals and the effect of age and oocytes numbers (and thereby estradiol levels) on EMT, microinjuries induced by surgical procedures (caesarean section, curettages) may also play a role. It has been suggested (Azumaguchi et al., 2011) that the EMT in the follicular and luteal phase of a natural cycle is strongly associated with the number of previous curettages.

A limitation of the present analysis is that EMT was only assessed on day of embryo transfer and that further confounders (e.g. previous curettages) for the occurrence of thin endometrium were not documented. Furthermore, endometrial lining pattern and/or subendometrial Doppler flow have not been assessed and, accordingly, the conclusions of this work are limited to only the thickness of the endometrium.

Strengths of the analysis are the large sample size, the broad inclusion criteria (e.g. wide range of ages and ovarian response) and that complete, prospectively collected study data were available allowing evaluation of the association of EMT with IVF outcome while controlling for known confounders.

In conclusion, the study findings imply that EMT on day of embryo transfer should be ignored over a wide range of measurements (e.g. 22 mm) when monitoring IVF treatment. Interventions to correct thin EMT have little rational basis and should be abandoned until contrary evidence arises.

### Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

### Acknowledgements

We acknowledge the work of all participating centres in 07EU/Prg06 and 07USA/Prg05.
Authors’ roles

G.G. wrote the study concept, participated in the analyses, reviewed the analyses, created the figures and wrote the first draft of the article. S.T. did the statistical work and reviewed the final version of article. B. B. participated in the concept of the study, the data analyses and data interpretation and revised the article. The final article and order of authorship has been approved by all authors.

Funding

The study was supported by IBSA Institut Biochimique SA, Switzerland.

Conflict of interest

G.G. has received personal fees and non-financial support from MSD, Ferring, Merck-Serono, Finox, TEVA, IBSA, Glycotope, Abbott, Gedeon-Richter as well as personal fees from VitroLife, NMC Healthcare, ReprodWissen, BioSilu and ZIVA. S.T. and B.C. are employees of IBSA.

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