The shortcomings of an unphysiological triggering of oocyte maturation employing hCG

Yding Andersen C, DMSc[1,2,7], Kelsey T, PhD[3], Mamsen LS, PhD[1], Vuong LN, MD, PhD[4,5,6]

1. Laboratory of Reproductive Biology, The Copenhagen University Hospital, Copenhagen, Denmark
2. Faculty of Health and Medical Science, Copenhagen University, Copenhagen, Denmark
3. School of Computer Science, University of St Andrews, St. Andrews, Scotland
4. Department of Obstetrics and Gynecology, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam;
5. IVFMD, My Duc Hospital, Ho Chi Minh City, Vietnam;
6. HOPE Research Center, My Duc Hospital, Vietnam.
7. Corresponding author: Claus Yding Andersen, Laboratory of Reproductive Biology, Section 5712 Juliane Marie Centre for Women, Children and Reproduction, University Hospital of Copenhagen Blegdamsvej 9, DK-2100 Copenhagen, Denmark,
Phone: +45 3545 5822; Fax: +45 3545 5824; E-mail: yding@rh.dk

Capsule: The widely used hCG trigger to induce final maturation of follicles during ovarian stimulation causes abnormal hormonal conditions in the luteal phase, which may exert negative impact on implantation potential.

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Abstract

Final maturation of follicles has, in connection with ovarian stimulation and infertility treatment, traditionally be achieved by administration of a hCG bolus trigger of 5.000 to 10.000 IU. This trigger serves two purposes: 1) induce oocyte maturation and 2) serve as luteal phase support due to its long half-life. It now appears that the hCG bolus trigger is unable to support both these two purposes optimally. In particular, following a hCG trigger, the early luteal phase is hormonally abnormal and different from conditions observed in the natural menstrual cycle: 1) The timing of the initiation of hCG and progesterone rise is much faster after a hCG trigger than in natural menstrual cycle 2) the maximal concentrations of hCG and progesterone considerably exceed those naturally observed 3) The timing of the peak progesterone concentration following an hCG trigger is advanced several days compared to the natural cycle. Furthermore, the hCG trigger without any FSH activity may induce oocyte maturation less efficiently than the combined LH and FSH surge normally seen.

Collectively, the endometrium is likely to be advanced following an hCG trigger and implantation potential is probably not optimal. The precise effect on pregnancy rates following the different progressions of hCG and progesterone concentrations during the early luteal phase has not yet been determined, but more individualized methods using more physiological approaches are likely to improve reproductive outcomes.

Introduction

Right from the beginning of the IVF era in the early 1980s, one of the few tools used for IVF treatment that has remained unchanged over time is the use of a bolus trigger of human chorionic gonadotropin (hCG) to achieve final maturation of follicles as a surrogate for the midcycle surge of gonadotropins. A bolus of hCG between 10.000 and 5.000 IU administered 36 hours before oocyte collection or timed ovulation has remained the gold standard, has persisted unchanged in nearly all protocols until recently, and has literally been applied to millions of women. Compared to the natural menstrual cycle in which LH concentrations normally reach 40—60 IU/L during the midcycle surge of gonadotropins, the hCG trigger results in much higher concentrations of hCG (i.e. LH-like activity) especially during the first 48 hours after injection, where concentrations often will reach 130 to 300 IU/L or even more, depending on the dose of hCG administered and BMI of the patient (1–3). As a result, hCG triggering is effective in securing maturation of oocytes enclosed in pre-ovulatory follicles irrespective of whether or which type of ovarian stimulation has been used. In addition, the relatively long half-life of hCG, which combined with the relatively large dose administered, results in the persistence of substantial levels of hCG during the first half of the luteal phase (2,4). Thus, the hCG bolus trigger serves one additional purpose by securing stimulation of the corpora lutea (CL) to secrete progesterone whilst hCG concentrations remain at a sufficient magnitude after the trigger injection (1). The strong luteal support from the hCG in the early luteal phase is also the reason behind the risk of ovarian hyper stimulation syndrome (OHSS) being a serious side effect of ovarian stimulation, illustrating that it may be difficult to find an appropriate luteal stimulation for each individual woman.

In essence, the hCG bolus trigger serves two purposes, first to secure maturation of oocytes and secondly to secure gonadotropin stimulated progesterone secretion prior to implantation.

Recent studies have suggested that these two purposes of the hCG bolus trigger appear to be a compromise between these two intended functions. It is now clear that hCG trigger leads to hormonal conditions in the early luteal phase which are unphysiologically high, are quite different from the natural menstrual cycle, and may not lead to optimal chances for successful reproductive outcomes in connection with ART (3). In fact, a more physiological and personalized luteal phase support may on one side improve reproductive outcomes and on the other side reduce the risk of OHSS (5). Actually, both oocyte maturation and stimulation of the early luteal phase secretion of progesterone as induced by the hCG bolus trigger, may on their own be optimized to improve ART outcome. The combined optimized functions have now changed our perception of both how oocyte maturation and luteal phase support should optimally be performed (6). This new knowledge has not yet been fully translated into clinical practice and considerable optimizations are still required, but this new knowledge already results in reproductive outcomes equally good as previously, with

fewer side effects and more options for individualizing treatment. The aim of current review is to highlight this new information and demonstrate how the hCG bolus trigger induces unphysiological conditions, which may induce less optimal reproductive outcomes for currently used ART procedures.

Function of the mid-cycle of gonadotropins or the bolus trigger of hCG

Ovulation initiated by the midcycle surge of gonadotropins or by a bolus trigger of hCG induces several distinct events which are separately regulated but need to be tightly coordinated and synchronized to secure optimal chances for successful reproduction. First, oocytes need to receive proper stimulation to resume meiosis and advance to the metaphase II of the second meiotic division, thereby becoming prepared to sustain fertilization and further development. Secondly, a series of inflammatory responses within the follicle needs to be initiated to secure the structural changes leading to follicle rupture and expulsion of the competent oocyte from the follicle to the oviduct for further development, while mechanisms to terminate this inflammation immediately after the oocyte release are required to allow proper development of the corpus luteum (CL) (7–10). In addition, transformation of the both the granulosa and theca cells into lutein cells with primary focus on progesterone secretion entails considerable remodeling of the cells, their steroidogenic apparatus and their niche.

Oocyte maturation

The hCG trigger only contains LH-like activity and lacks any FSH activity, which contrasts with the natural situation in which a FSH peak is also released during the midcycle surge of gonadotropins, albeit at a somewhat lower magnitude as compared to the LH concentration (11). The role of FSH during the natural midcycle surge is not completely clear, but FSH induces LH receptor formation on granulosa cells, which during the ovulatory process are of upmost importance for oocyte maturation and for optimizing subsequent CL function. Whereas FSH are mandatory for oocyte maturation in mice (12,13) and important in several animal species, FSH appears to be less important in human oocyte maturation especially in connection with ovarian stimulation where hCG alone with good efficacy secures MII transition of oocytes in preovulatory follicles. This is likely to reflect that ovarian stimulation leads to increased levels of FSH throughout most of the follicular phase – often with concentrations of FSH in the range of those seen during the midcycle surge. Thus, the FSH induced actions including LH receptor formation on granulosa cells may already have been achieved prior to the midcycle surge and as a consequence the lack of FSH released is of less importance.

In contrast, in connection with the use of the agonist trigger for final maturation of follicles in connection with ovulation induction, where the endogenous stores of gonadotropins are mobilized, both LH and FSH are

released from the pituitary during ovulation induction. Several clinical studies have found a small increase in the number of MII-oocytes obtained as compared to the hCG trigger (14–18) and to cumulus expansion, suggesting that even in connection with ovulation induction the combined action of LH and FSH in the actual ovulatory process is of importance.

Although regulation of oocytes maturation has been studied intensively in rodents and animal species during the last 50 years, the regulatory pathways operating during human oocyte maturation is only partly resolved, and it is clear that many of the mechanisms of importance in animal models are not regulated the same way in humans and indeed it appears that several different regulatory pathways are active during the ovulatory process in humans at the time of *in vivo* oocyte maturation (10,19).

It is now clear that human oocyte maturation takes place during the first 17-19 hours of the ovulatory process itself (20) and recent studies have shown, on both protein and gene levels, that several processes advancing oocyte maturation are active during this period including some of which are enhanced by FSH including synthesis of inhibins (10,19). The multitude of signal transduction pathways, which individually may advance human oocyte maturation *in vivo*, demonstrates the complexity and suggests that the bolus hCG trigger may only provide part of an optimal response.

Characteristics of the hCG bolus trigger that induces unphysiological conditions

There are several characteristics of the hCG trigger that results in conditions during ovulation and in the early luteal phase that contrast with conditions observed in the natural cycle, leading to unphysiologically high hormonal conditions. These include a rapid rise of hCG immediately after administration of the hCG trigger (fig 1.; table 1), high maximal concentrations of hCG, and a high prolonged exposure to hCG in the early luteal phase (fig. 1; table 1) (1–4).

We have recently performed studies on 160 women undergoing ovarian stimulation following the antagonist protocol, receiving 6.500 IU rhCG for final maturation of follicles (3,21). All these women underwent “freeze-all” for various reasons and did not receive fresh embryo transfer. Consequently, they were not given luteal phase support in any form, but the concentrations of hCG and progesterone were recorded at 0, 12, 24 and 36 h after ovulation trigger and then daily during the first six days after OPU. Thus, these studies for the first time provided a detailed information on the concentration of hCG during the first half of the luteal phase in a clinical setting (3). It is interesting to notice the initial rise of hCG after the bolus injection in comparison to the natural menstrual cycle (fig.1; table 1). The maximal concentration of hCG is observed 19 hours after

injection (fig.1) and after 12 hours the concentration of hCG is on average 126 IU/L (table 1). During the natural menstrual cycle, the peak levels of LH occurs 24 hours after initiation of the midcycle surge at concentrations of around on average 40—60 IU/L, with concentrations being around 25 IU/L after 12 hours (11,22,23). Thus, 12 hours after initiation of ovulation induction there is on average a five times higher concentration of LH-like activity following a hCG bolus trigger as compared to the natural menstrual cycle. This results in a much stronger push for progesterone synthesis in connection with ovulation as demonstrated in a study comparing the agonist trigger with the hCG trigger where concentrations of progesterone at OPU were three times higher in the hCG group as compared to agonist trigger group (5). As a consequence, the endometrium will be exposed to high transformative concentrations of progesterone much earlier during the luteal phase in connection with the hCG trigger as compared to the agonist trigger, resembling the natural midcycle surge, suggesting that early advancement of the endometrium may take place. This potentially attenuates the implantation potential.

In the data set from Vuong and coworkers (3) the peak concentration was reached after 19 h at 140 IU/L. This obviously provides a very strong luteotropic signal, which causes a hefty stimulation of CL to secrete progesterone and other CL related hormones. Actually, considering the area under the curve of hCG and LH concentrations in connection with a bolus trigger of 6.500 IU and a natural mid-cycle surge, a five times stronger signal is exerted by the hCG trigger during the first 48 h, emphasizing the strong luteotropic signal exerted by hCG (fig.1). However, the range of hCG concentrations are wide, spanning from 40 to more than 300 IU/L (fig.1. and table 1) (3). Thus, for the hCG trigger to be effective for all patients, giving that the lower range of peak concentrations starts at 40 IU/L, which corresponds to the average LH levels observed during the natural menstrual cycle, a bolus trigger of 6.500 IU is required. This, on the other hand, results in a relatively large number of women having high concentrations receiving a strong luteotropic signal – perhaps too strong when the day of peak progesterone concentration is also considered (i.e. OPU+4, see next section). Taken together, at the expense of one hCG dose fits all, many women will receive a very strong signal for stimulation of the CL to produce progesterone, which may result in advancement of the endometrium too early and provide less optimal conditions at the time of normal implantation.

One additional effect of the bolus trigger of hCG is to secure sustained concentrations of hCG during the first half of the luteal phase. Originally, the intended purpose of the hCG trigger was to secure a continued stimulation of CL due to pituitary inactivation. From table 1 it can be seen that median hCG value on OPU+4, OPU+5 and OPU+6 is 8.2, 4.6 and 2.4 IU/L, respectively. Provided that a physiological concentration of LH-like activity (i.e. either LH or hCG) is considered to be around 5 IU/L during the luteal phase, then 17%, 59% and 89% of the whole cohort of women from this study have a concentration below 5 IU/L on OPU+4, OPU+5 and OPU+6, respectively. As consequence, almost all women experience a reduced stimulation for

progesterone secretion on OPU+4—6, which coincides with the day of implantation and where the secretion of hCG from the implanting embryo is still very limited and without effect on the CL.

Collectively, at the crucial time of implantation at OPU+6—7, the stimulation of progesterone secretion is at a nadir in connection with the hCG trigger (4). In contrast, in the natural menstrual cycle the concentration of progesterone peaks at this time (11,22,23). This highlights the benefits of exogenous administration of progesterone for luteal support, which is now commonly used in connection with ovarian stimulation. The exogenous progesterone serves as a remedy for maintaining levels of progesterone sufficiently high with a reduced contribution from the CL due to reduced hCG stimulation, facilitating a better chance of a successful outcome of treatment. However, most forms of exogenous administration of progesterone only provide a relatively modest increase in the circulation concentrations of progesterone (15).

Characteristics of the unphysiological progesterone conditions induced by the hCG trigger

Progesterone is the essential hormone to ensure the secretory transformation of the endometrium to allow embryo implantation and maintenance of early pregnancy (24). After implantation during the mid-luteal phase, secretion of hCG by the implanting blastocyst secures continued secretion of progesterone and function of the CL (25). Use of the hCG trigger in IVF cycles results in supraphysiological concentrations of progesterone during the early luteal phase (26–28) and in IVF it is well documented that the luteal progesterone profile is substantially different from the progesterone profile in natural cycles, where peak progesterone concentrations usually occur at approximately 6–8 days after ovulation to coincide with the expected time of implantation (fig.2) (29–32). Actually, there are three key figures of progesterone secretion in the early luteal phase, which following a hCG trigger stand out as very different from the natural conditions. First, the speed by which progesterone rises in circulation after administration of the ovulation trigger. Second, the peak concentration of progesterone reached after the hCG trigger. Third, the day the peak concentration of progesterone is reached. However, the progesterone concentrations reflect the hCG levels only to a limited extent (3,21).

In the luteal phase of the normal cycle, a progesterone rise beyond 25 nmol/L is considered enough to show ovulation, support implantation and is normally reached during the mid-luteal phase. However, data on early luteal phase hormone levels are rare in women receiving a hCG bolus trigger. In addition, initial studies evaluating the early luteal phase steroid profile after hCG trigger have been limited by small patient populations (33–35). However, the speed by which progesterone rises after ovulation induction is nicely illustrated by recent data from (21) which included a total of 160 women receiving 6.500 IU for final maturation of follicles and no exogenous luteal phase support (table 1). On average the peak progesterone concentration during the luteal phase in natural cycles was exceeded at between 12--24 h after ovulation

induction (21), in contrast with the natural cycle where concentrations only slowly start to increase and remained on average below 5 nmol/L during the first 24 h after ovulation triggering (fig. 2) (11,22,23).
This massive increase in progesterone in the early luteal phase in connection with the hCG trigger reflects both the multiple pre-ovulatory follicles usually available during ovarian stimulation and the massive exposure to hCG. In addition, pre-ovulatory follicles resulting from ovarian stimulation with exogenous FSH administration are probably hyper-sensitive to hCG, since they have been exposed to supra-physiological concentrations of FSH, which have induced LHR expression on granulosa cells to a higher degree than during a normal cycle (36).
Therefore, the premature early luteal phase rise in progesterone that occurs after ovarian stimulation with exogenous gonadotropins and hCG trigger is likely to result in advancement of the implantation window, causing asynchrony between the embryo and the endometrium, which may contribute to reduced implantation rates (37–39).

It is well known that progesterone concentrations after a hCG trigger exceeds those of normal menstrual cycles, but only limited systematic data are available of the maximal concentration during the early luteal phase. Further, there is also little information on which day during the luteal phase, peak concentrations of progesterone will occur. The recent studies from Vuong and colleagues (3,21), demonstrated that the average peak concentration of progesterone occurred at OPU+4 days, but with considerable variability between patients (table 2; fig. 2). Almost one in five patients had already experienced a peak progesterone concentration on OPU+2-3, and only one in seven had maximal concentrations on OPU+6, showing that for a total of 85% of women experienced their highest concentration before the period in which the peak was expected to be reached during a natural menstrual cycle (table 2). The mean percentage decrease in progesterone from the day of peak concentration to OPU+6 was relatively constant on OPU+3, OPU+4 and OPU+5 with levels of 58%, 55% and 48%, respectively, showing a relatively steep decline from OPU+5 to OPU+6 in patients peaking on OPU+5 with concentrations on average being halved (table 2).
These studies also provide information on the peak progesterone concentrations, which on average reached 426 nmol/L with a median value of 340 nmol/L on day OPU+4. This peak concentration is approximately ten times higher than that seen during the natural luteal phase and probably reflects that on average 11 follicles exceeding 14 mm at ovulation induction were monitored (fig. 2) (21). This suggests that each individual CL produced progesterone at a magnitude similar to the natural cycle with only one CL, but the cumulative output of progesterone during ovarian stimulation was 10 to 15 times higher. It is noticeable that the peak concentration of progesterone is similar irrespective of which day during the early luteal phase it peaks (table 2). This may reflect that the CL are producing progesterone with maximal output during strong hCG

stimulation in the early luteal phase, but the sensitivity (i.e. the LHR expression) of the granulosa-lutein cells may differ between patients who peak on OPU+2 or OPU+3 having a higher sensitivity.

The range of progesterone concentrations is massive, spanning from 79 to 805 nmol/L on OPU+4 (with the other days showing similar wide ranges) (fig.2). Further, it is interesting that the peak concentration of progesterone occurs when the corresponding average concentration of hCG is just 9 IU/L after having peaked at 140 IU/L (fig 3). This probably demonstrates that a relatively modest concentration of hCG is sufficient to stimulate maximal production of progesterone by the CL, in line with in a previous study including women undergoing IVF treatment and ovarian stimulation where the progesterone concentration on OPU+6 reached 330 nmol/L with CL only being stimulated with concentrations of hCG in the physiological range of LH (5).

How these highly unphysiological conditions affects the endometrium, its receptivity and the ability to support implantation has not been clarified in detail.

Consequences of the progesterone concentrations peaking earlier than expected in connection with a bolus trigger of hCG.

A number of studies have tried to associate the midluteal phase progesterone concentrations (i.e. OPU+6) with ongoing pregnancy rates and the reproductive outcome (15–17,40). Some studies have found that a lower threshold concentration of progesterone predicts an enhanced early pregnancy loss and reduced ongoing pregnancy rate (4); other studies also determine a maximal threshold value beyond which the reproductive outcome become reduced (41), while others fail to observe this. However the recent information from Vuong and coworkers (3,21) showed that on average the peak concentration of progesterone occurs on OPU+4, with two thirds of the patients experiencing a fall of progesterone concentration from OPU+4 to OPU+6 and more than 40% of patients experiencing a more than a 50% reduction in progesterone concentration. Hence it becomes difficult to determine which parameters are of most importance for successful reproductive outcome. It could be that the reproductive outcomes for those patients who experience the most pronounced reduction from peak progesterone to OPU+6 will be negatively affected, with similar reasoning applying to the around 20% of the patients that already experience a peak progesterone concentration on OPU+2 or OPU+3 – irrespective of what value the absolute concentration of progesterone had on the day of implantation (i.e. OPU+6). In essence, these new data open up a whole set of questions and motivate a new series of studies on the luteal phase to evaluate the unphysiological effects of the hCG bolus trigger and the potential for an optimized luteal phase support and improved reproductive outcomes.

Lessons from available data

There is a good body of knowledge regarding serum progesterone levels during the midluteal phase showing that the implantation process is sensitive to variability in mid-luteal serum progesterone concentrations (42), and that low mid-luteal phase progesterone levels have a negative impact on pregnancy outcomes in both fresh and frozen IVF cycles (41,43). From the current data it is clear that luteal phase support might be more effective if the timing and dosage are adjusted for each patient as compared to the currently used schemes for luteal phase support in connection with the use of the hCG trigger (44–47). Individualization of the FSH dose during ovarian stimulation is a common approach, and it is likely that a tailored luteal phase support could contribute to further improvements in the implantation rate during IVF. This individualized approach is important because a recent clinical trial showed inter-individual variation in the response to a bolus hCG trigger dose (3). Both the dosage and timing of luteal phase support are likely to be important. The early luteal phase data suggests that the early progesterone rise shortly after hCG trigger might be a trigger for endometrium damage (3). Progesterone concentrations in the late follicular phase have been identified as a significant predictor of progesterone concentrations at all time points up to the day after oocyte pick-up (3). This confirms that late follicular phase progesterone levels reflect the sensitivity of follicles to LH, which in turn determines the early luteal phase response. Of all the measurements taken in the recent study (3), serum progesterone levels at 12 and 24 hours after administration of hCG trigger were the best predictors of progesterone concentrations at all other time points up to OPU+6 (the final measurement in the study). Therefore, serum progesterone values measured at 12 and 24 hours could be used in clinical practice to determine the course of progesterone concentrations during the subsequent luteal phase, and to guide individualized strategies to optimize luteal phase support. Another recent study has suggested that early luteal phase progesterone levels (on day 2-3 after oocyte pick-up) might be associated with reproductive outcomes in women undergoing IVF with fresh embryo transfer who received luteal support with oral progesterone (48).

In addition to variable progesterone concentrations after hCG trigger, there is also wide inter-individual variability in early luteal phase hCG concentrations (21). Although it was difficult to determine factors that predicted low levels of hCG after a bolus trigger dose in this retrospective analysis, body mass index (BMI) appeared to be important – hCG levels were higher when BMI was lower (21). A cluster analysis performed as part of the study showed that patients who had the lowest serum concentrations of hCG and progesterone at 12 hours after hCG trigger had a significantly higher BMI, significantly shorter duration of stimulation, and significantly lower anti-Müllerian hormone levels and numbers of follicles of ≥11 and ≥14mm in diameter compared with the other three clusters (21). These findings suggest that some patients may have suboptimal stimulation of the CL due to low levels of hCG, potentially impacting on the outcomes of fertility treatment.

Based on the predictors studied, it would appear that women with a higher BMI might need additional luteal phase support due to more rapid decline in hCG levels compared to those with a lower BMI (21).
The value of understanding the early luteal phase hormone profile may be an improved ability to predict which factors are important for the mid-luteal phase progesterone concentrations. In turn, this facilitates the development of strategies to optimize serum progesterone levels throughout the luteal phase, hopefully improving the implantation rate. More detailed information on hormone levels during the critical luteal phase period are needed, and this is an important area for future research. It would also be interesting to determine comparative pregnancy rates in patients with earlier versus later peak progesterone levels after hCG trigger, and to understand whether it is the absolute value of progesterone or the rate of decline in progesterone levels that is important. What we know now is that progesterone must appear at the right time and in the right amount to facilitate successful implantation: not too early, not too late, not too low.

Conclusions

The hCG bolus trigger has a major impact on at least three conditions causing unphysiological circumstances of importance for implantation and reproductive outcome:

1) The hCG bolus trigger induces a very rapid increase in progesterone concentrations, which after 12-24 hours exceeds those normally seen as peak luteal phase concentrations in the natural menstrual cycle potentially advancing endometrial receptivity.
2) The peak concentration of progesterone is on average advanced two days as compared to the natural luteal phase, with almost 20% of patients experiencing maximal concentrations two or three days after oocyte pick-up.
3) The peak concentration of progesterone is almost ten times higher after ovarian stimulation and the hCG trigger compared to the normal luteal phase. A large fraction of women experiences a pronounced drop in the progesterone concentration from the day it peaks until the day of expected implantation.
4) There is a huge variability between patients in the progesterone concentrations during the early luteal phase and the sensitivity towards hCG stimulation differs considerably.
5) On five to six days after oocyte pick-up the majority of women experience levels of hCG below the physiological concentrations of LH during the luteal phase.

Despite the widespread use of a bolus trigger of hCG for final maturation of follicles and its proven clinical efficacy over the years, it clearly induces highly unphysiological conditions. This has been accepted as a compromise between the two functions undertaken the hCG trigger: induction of oocyte maturation and

luteal phase support. The impact on implantation and reproductive outcomes in women undergoing ovarian stimulation with development of multiple follicles is not yet clear but is likely to be individually optimized and further research in this area is needed.

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Figure legends:

Fig. 1. Dose response curve of hCG concentrations after injection of a bolus trigger of 6.500 IU until 6 days after oocyte pick-up in 160 women receiving a bolus trigger of 6.500 IU hCG after ovarian stimulation (21). Data on the natural midcycle surge of LH based on average data published in (11,22,23). Data are Mean± 1SD.

Fig. 2. Concentrations of progesterone from the time of ovulation trigger until 6 days after oocyte pick-up in 160 women receiving a bolus trigger of 6.500 IU hCG after ovarian stimulation with no exogenous administration of progesterone. Individual measurements are indicated with dots – data are from (3). Data on the natural luteal phase progesterone concentrations based on average data published in (11,22,23). Data are Mean± 1SD.

Fig. 3. The combined data of fig. 1 and fig. 2 showing the average hCG— and corresponding progesterone concentrations in 160 women receiving a bolus trigger of 6.500 IU hCG after ovarian stimulation with no exogenous administration of progesterone. Data from the natural luteal phase progesterone concentrations are also depicted based on average data published in (11,22,23). Data are Mean± 1SD.