hCG as luteal phase support without exogenous progesterone administration: Mathematical modelling of the hCG concentration to hypothesize on an optimised luteal phase support

Running title: Optimisation of luteal phase support using low dose hCG

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Abstract

For the last two decades exogenous progesterone administration has been used as luteal phase support (LPS) in connection with controlled ovarian stimulation (COS) combined with use of the hCG trigger for final maturation of follicles. The introduction of the GnRHa trigger to induce ovulation showed that exogenous progesterone administration without hCG supplementation was insufficient to obtain satisfactory pregnancy rates.

This has prompted development of alternative strategies for LPS. Augmenting the local endogenous production of progesterone by the multiple corpora lutea has been one focus with emphasis on one hand, to avoid development of ovarian hyper stimulation syndrome and on the other hand, to provide adequate levels of progesterone to sustain implantation.

The present study evaluates the use of low dose hCG for LPS support and examines the potential advances and disadvantages. Further, based on the pharmacokinetic characteristics of hCG, mathematical modelling of the concentration profiles of hCG during the luteal phase has been evaluated in connection with several different approaches for hCG administration as LPS. It is suggested that the currently employed LPS provided in connection with the GnRHa trigger (i.e. 1.500 IU) is too strong, and that daily low dose hCG administration is likely to provide an optimised LPS with the current available drugs.

Introduction

A bolus injection of human chorionic gonadotropin (hCG) has been consistently used for final maturation of oocytes since the introduction of IVF treatment, and is considered the gold standard in connection with assisted reproduction techniques (ART). However, the hCG trigger is associated with a high risk of ovarian hyper stimulation syndrome (OHSS) especially in high responder patients (Mathur *et al*., 2000; Papanikolaou *et al*., 2006; reviews: Aboulghar, 2009; Papnikolaou *et al*., 2010). Moreover the massive stimulation of progesterone production in the early luteal phase due to the supra-physiological levels of LH-like activity differs profoundly from the natural progesterone profile during the luteal phase (Fauser *et al*., 2002). With the hCG trigger, progesterone levels peak shortly after oocyte pick up (OPU) and prior to the mid-luteal phase, when progesterone in the natural menstrual cycle reaches its peak concentration. It has been suggested that this early rise in progesterone may lead to an advancement of the endometrium and reduced implantation (Yding Andersen et al., 2015).

In recent years the GnRHa trigger has been used as an alternative to the hCG trigger in patients following the antagonist protocol (Humaidan et al., 2005: 2006: 2013). The GnRHa trigger induces an endogenous surge consisting of both FSH and LH, but at a lower magnitude of LH-like activity than that of the both the hCG trigger and the area under the curve of the natural mid-cycle surge (Fauser et al., 2002). As the magnitude of the ovulation trigger is closely associated with the risk of OHSS, the GnRHa trigger combined with a 1.500 IU dose of hCG given at OPU, effectively reduces, but does not eliminate, the risk of OHSS despite multi-follicular development [(reviews: Aboulghar, 2009; Papnikolaou et al., 2010). This feature of the GnRHa trigger alone has already paved the way for a widespread use of this concept in clinical practise and especially in high responder women, who may receive a fresh transfer or be used in connection with a freeze-all strategy (Humaidan et al., 2011). The GnRHa trigger concept has found use combined with the freeze-all strategy because low risk of OHSS, no luteal phase support (LPS) is required and, the efficacy by which oocytes complete final maturation only exhibit modest differences between the hCG trigger and the GnRHa trigger. However, many clinics do not have an optimized vitrification program and many women request a fresh transfer after a burdensome treatment and a number of clinics still focus on optimizing LPS and reducing the risk of OHSS.

One important difference between the GnRHa trigger and the hCG bolus trigger is the effect on the early luteal phase (Yding Andersen et al., 2015; Humaidan et al., 2013). In contrast to the hCG trigger, the GnRHa trigger is without stimulatory effect on the newly formed corpera lutea (CL). Thus one pronounced characteristic of the GnRHa trigger is that it separates the two events traditionally undertaken by hCG, specifically induction of final follicular maturation and maintenance of the CL for early (LPS) (Humaidan et al., 2005: 2006: 2011: 2013).

The GnRHa trigger exerts a direct down regulation of gonadotropin release from the pituitary, resulting in an insufficient amount of LH that is vital during the early luteal phase and necessary for continued function of the CL. Therefore, the GnRHa trigger used without enhanced LPS consisting of either exogenous LH-like activity or substantial progesterone supplementation yields lower pregnancy rates than the hCG trigger [Humaidan et al., 2005; Kolibianakis et al., 2005]. The lower pregnancy rates of the GnRHa trigger appear to be associated to a low mid-luteal phase progesterone level and probably reflect too low stimulation of the CL. Several studies have now suggested that in connection with COS the mid-luteal phase concentration of progesterone should exceed 100 nmol/l in order to reduce the risk of early pregnancy loos and augment reproductive outcome (Liu *et al.,* 1995; Yovich *et al*., 2015; Yding Andersen & Andersen, 2014). This concentration of progesterone is difficult to obtain through vaginal or im. administration alone and necessitates a direct stimulation of the CL with either rLH or hCG. The hCG bolus trigger last until shortly before the mid-luteal phase and does usually result in levels of progesterone exceeding 100 nmol/l (Yding Andersen et al., 2015). Therefore, in order to develop the GnRHa trigger further, evaluation and understanding of the current methods and concepts for LPS are gaining interest. Based on mathematical modelling of the pharmacokinetics of hCG the focus of this paper is to discuss current dogmas on LPS and to suggest new ways of performing LPS including the exogenous progesterone-free concept, which recent clinical experience suggest may be performed using low dose daily hCG supplementation (see below).

The use hCG for luteal phase support

The potential use of hCG for LPS in addition to the hCG bolus trigger has been considered an option for many years. However, a Cochrane analysis from 2011 on LPS for ART cycles (van der Linden et al., 2011) concluded that there was no significant effect in favour of exogenous progesterone plus hCG as compared to progesterone alone for LPS. This analysis found that hCG,

or hCG plus progesterone, was associated with a higher risk of OHSS and concluded that the use of hCG should be avoided (van der Linden et al., 2011). Further it was concluded that exogenous progesterone seemed to be the best option for LPS providing better pregnancy results.

However, a modest four to five hundred patients were in total included into this arm of the Cochrane analysis on this aspect. Further, the dose of hCG given as LPS in these studies is nowadays considered far too high. For example, in the study by Kupferminc and co-workers (Kupferminc et al., 1990) 2500 IU hCG was given three times during the luteal phase in addition to the bolus trigger of hCG. The other studies included in the Cochrane analysis also used relatively large bolus injections of hCG two or three times during the luteal phase. By today's standard this is a very strong LPS, and figure 1 illustrates the levels of hCG expected during the luteal phase calculated based on the reported half-life of hCG (Trinchard-Lugan et al., 2002); the average levels of hCG range between 40 – 80 IU/L during the entire length of the luteal phase. This is around ten times higher than the LH concentration observed during the natural menstrual cycle (i.e. 4-10 IU/L). It is therefore not surprising that the incidence of OHSS was increased. We have recently shown that much lower levels of hCG can adequately support the function of the CL (Yding Andersen et al., 2015). Even conditions observed during the natural menstrual cycle in which levels of LH remain between 4 – 8 IU/L during the entire luteal phase are sufficient to stimulate CL secretion of progesterone significantly more compared to the use of the traditional hCG trigger and vaginal progesterone (Yding Andersen et al., 2015).

Furthermore there are two lines of circumstantial experimental data that suggest, contrary to previous belief, that pharmacological levels of hCG during the early/mid luteal phase actually may exert a negative impact on implantation. First the demonstration of functional LHR in the endometrium with ERK 1/2 signalling in both women undergoing IVF treatment and women in their natural menstrual cycle (Evans & Salamonsen, 2013) may affect implantation. Under the title "Too much of the good" this study found that chronic exposure to normal hCG *in vitro* mediated a down-regulation and internalization of LHR on endometrial epithelial cells (Evans & Salamonsen, 2013). Exposure to hCG for 3–5 days abrogated ERK 1/2 phosphorylation, and attenuated adhesion to extracellular matrices and changes in tight junction integrity in response to an acute high dose of hCG. Endometrial epithelial LHR staining was significantly lower in women who did not become pregnant (long agonist protocol) versus the natural menstrual cycle ($P<0.05$)

suggesting that functional LHR signalling is of importance for facilitating implantation (Evans & Salamonsen, 2013).

Another line of experimental data relate to detailed studies on the molecular structure of hCG, which have revealed several different molecular forms of hCG that appear to exert different functions (Cole, 2012a). Especially, hyperglycosylated hCG (H-hCG) appears to play a distinct role during implantation (Cole, 2012a). Hyperglycosylated hCG is a form of hCG that becomes considerably more glycosylated than normal hCG, although the number of glycosylation sites remains similar to that of normal hCG. The increased glycosylation makes the H-hCG fold in a way different to normal hCG, leading to biological properties that normal hCG does not possess. In addition to activating the LHR, the H-hCG also acts as a TGF-β antagonist (Cole, 2012c). When normal and H-hCG are measured during normal implantation, almost all the hCG secreted from the implanting blastocyst is H-hCG and none is normal hCG, while normal hCG only takes over later on during pregnancy. It has been suggested that it is the deficiency of H-hCG that causes incomplete blastocyst implantation or pregnancy failures, biochemical pregnancies and miscarriages (Cole, 2012b). It may be speculated that just when the implanting embryo starts to secrete almost exclusively H-hCG in very small quantities, the presence of normal hCG of around 40 – 80 IU/L (similar to those studies included in the meta analysis of LPS, fig.1) attenuates successful implantation because it may interfere with the specific actions mediated by H-hCG.

The practical implication of this is that stimulation of progesterone secretion from the CL should be performed with low physiological concentrations of LH-like activity, which has recently been shown to be feasible using hCG (Yding Andersen et al. 2015) and previously using r-LH (Pappanikolaou et al., 2011). Levels of hCG associated with the use of the hCG bolus trigger level-off and become very low during the mid-luteal phase, usually in the lower end of the physiological range (Yding Andersen & Andersen, 2014). In addition the bolus hCG trigger elicit a progesterone peak in the early luteal phase starting to decline prior to the time of implantation.

Taken together, circumstantial evidence indicates that supra-physiological levels of hCG during the mid-luteal phase may interfere with successful implantation. Furthermore, increased concentrations of hCG augment the risk of OHSS. On the other hand low physiological concentrations of hCG can accomplish sufficient stimulation of CL and provide support to further develop a LPS based on only low dose luteal phase hCG supplementation. We have recently

conducted a small RCT in which the only LPS provided in the test arm was a low dose of hCG of 125 IU daily in connection with a GnRHa trigger. This was compared that to the standard antagonist protocol using a bolus trigger of hCG for final maturation of oocytes and LPS in the form of conventional vaginal progesterone tablets (i.e. Crinone)(Yding Andersen et al., 2015). During the mid-luteal phase (i.e. day OPU+7) levels of hCG were on average twice as high in the GnRHa trigger/low-dose hCG group compared to the hCG trigger group but within the physiological range. The concentrations of progesterone were on average around twice as high in the GnRHa trigger/low-dose hCG group ($P<0.05$). Indeed, this study demonstrated that a low physiological concentration of LH-like activity will stimulate CL sufficiently, and that supra-physiological concentrations of hCG are not necessary. In retrospect this is perhaps not too surprising since one CL during the natural menstrual cycle normally produce around 30 nmol/l during the mid-luteal phase, then ten CL may, provided that sufficient LH-like activity is present, just produce ten times more. In this respect the physiology of the luteal phase is different from the follicular phase where FSH needs to be increased to stimulate multi-follicular development.

Local effect versus systemic effect of progesterone

The veins draining the ovary come in close contact with the uterus and the vessels leading to the uterus. Since the CL is the main source of progesterone, studies in sheep have demonstrated that the mean progesterone concentration in the ovarian vein draining a CL-containing ovary was 800-fold higher than mean jugular venous levels (Abecia et al., 1997). Furthermore, the veins from the contralateral ovary without a CL showed a mean progesterone concentration 30 times lower (ipsilateral: 3297 ±439; contralateral: 95 ±35 nmol/L; $P<0.001$), showing that the local concentration of progesterone that may exert an immediate local effect on the endometrium is considerably higher than that observed in the peripheral circulation. This point is further strengthened by the observation that the mean progesterone concentration in the uterine vein was approximately 30-fold higher than in jugular vein and similar in both uterine horns (Abecia et al., 1997).

Therefore stimulation of progesterone production within CL will most likely not only increase the circulating concentration of progesterone, but also augment the local effect on the uterus, which exogenous intramuscular or subcutaneous progesterone administered will not exert. It has been

claimed that vaginal progesterone tablets also exert an enhanced first pass stimulatory effect on the endometrium (Levine & Watson, 2000). However, there is a rather limited amount of progesterone that can be absorbed from the vagina, and the circulatory concentration peaks six to eight hours after administration at a modest 35-50 nmol/L meaning that the local uterine concentration has peaked even earlier (Cicinelli et al., 2000). When using the vaginal progesterone pessaries, the endometrium is therefore exposed to highly variable concentrations of progesterone. In contrast, the LH activity present in the luteal phase of natural menstrual cycle provides a far more constant exposure to progesterone, which although shows variations in the circulatory concentration of progesterone the fluctuations are much less pronounced (Filicori et al., 1984). It seems reasonable to expect that hCG present in similar concentrations would be exert a similar effect during the luteal phase.

Taken together, arguments are in favour of a local CL production of progesterone, which may exert a direct local effect on the endometrium and provide far higher concentrations of progesterone with just minimal stimulation rather than exogenous sources of progesterone.

Suggested introduction of a physiological low-dose hCG stimulation as luteal phase support

Various protocols have been considered in order to develop new and appropriate ways of performing LPS by use of gentle (i.e. physiological) stimulation with exogenous hCG. It is illustrative to observe the predicted course of hCG in circulation based on mathematical modulation of the pharmacokinetic information available (Trinchard-Lugan et al., 2002). The supplementary files show how the matemathical simulation of the hCG concentration has been performed (supplementary files). The mathematical hCG curves is obviously inferior to actual studies, which will be needed to substantiate these suggestions, but the material may help in developing protocols to be tested clinically. However, the predicted steady state concentration of hCG after daily administration of either 100 or 150 IU hCG as predicted by our model (fig.6) actually match exactly the steady state concentration observed in a clinical study in which patients received rec-FSH plus either 100 or 150 IU hCG daily for COS in a agonist protocol (Tuessen et al., 2012). These clinical data provides an external validation of the model, but we knowledge that generated data will provide average concentrations of hCG that require adjustment to individual

patient characteristics such as weight and body composition. In the future this highlights the ample possibilities for adjusting and individualising the dose administered.

The overall goal for LPS using exogenous hCG administration should be to provide sufficient LH-like activity to support the secretion of appropriate progesterone levels by the CL, until the endogenous hCG from the implanting embryo takes over. So, in essence, LH-like activity provided by LH plus hCG should be in the physiological range from the time of OPU until OPU+7/8. Different potential ways of performing LPS is given in figures 3-5, and additionally the predicted level of hCG seven days after OPU at the mid-luteal phase is calculated.

In the case where the traditional hCG bolus trigger (i.e. 5.000 – 10.000 IU) is used for final maturation of oocytes, there will be a gap of LH-like activity in the mid-luteal phase around the time of implantation as previously described (Yding Andersen et al., 2015; Andersen & Andersen, 2014). It may be envisaged that the gap of LH-like activity can be closed by providing a continued low dose of hCG (e.g. 500 IU) just prior to the mid-luteal phase (fig. 3). The hCG concentration will thus slightly increase and reach an average level of around 9 IU/L during the mid-luteal phase, providing continued CL stimulation until hCG produced by the pregnancy takes over. Furthermore, this approach could encourage a shift from an hCG trigger of 10.000 to 5.000 IU that theoretically should reduce the risk of OHSS. The exogenous level of hCG present in concentrations below 10 IU/L may only interfere with the implantation promoting effects of H-hCG to a limited extent, since LH normally present in the circulation in similar concentrations will also bind to the LH-receptor. Furthermore, the concentration of H-hCG in the vicinity of the implanting embryo is likely to be considerably higher. This option represents a possible way to optimize the traditionally used hCG bolus trigger, and is depicted in fig 3.

In connection with the GnRHa trigger, it is necessary to provide hCG activity starting from OPU until OPU+7/8 when the pregnancy derived hCG takes over. The now well-established bolus dose of 1.500 IU of hCG to stimulate CL function given at the time of OPU will result in hCG concentrations of around 30 – 50 IU/L tailing off to reach low concentrations around the mid-luteal phase. Administrating two bolus injections of 1.500 IU (day OPU and day OPU+5) in normally responding women (Humaidan et al., 2013) resulted in hCG levels around 40-60 IU/L during the mid-luteal phase, hence resulting in a slightly increased risk of OHSS (Fig. 2). However, as seen in

figure 2, both the first and the second dose of 1.500 IU hCG may probably be reduced without loss of sufficient stimulation of the CL.

An alternative could therefore be to reduce the dose of the bolus injection of hCG to 1.000 IU on the day of OPU followed by an injection of 500 IU on day OPU+5 (Fig. 4). This results in markedly lower concentrations of hCG, with concentrations in the physiological range around day OPU+7. However, peak concentrations of around 25 and 15 IU/L are observed shortly after injections, which may not be necessary for sufficient CL stimulation.

The provision of three bolus injections of hCG each of 500 IU in the early/mid luteal phase in connection with the GnRHa trigger to provide LPS has also been attempted (Castillo et al., 2010). This strategy results in hCG concentrations below 20 IU/L and will provide LH-like activity in the physiological range at OPU+7. The risk of inducing OHSS should be reduced although this particular study actually found a OHSS rate of 3.5% in this group with the majority being late onset OHSS (fig.5). This illustrates that levels of hCG should be constantly kept in physiological window to reduce the risk of OHSS.

In order to avoid any peaks of hCG activity, one approach is to provide low dose hCG injections daily (Tuessen et al., 2012). Based on the model, the course of hCG by daily injections of hCG (i.e. 100, 125 or 150 IU) is shown in fig. 6. It is seen that the steady state concentrations are around 6, 8 or 9.5 IU/L respectively, and each remains within the physiological range of LH-like activity throughout the luteal phase. It may be conceptualised that in order to obtain a quick increase in LH activity after OPU, different doses could be administered with, for instance, 150 IU for the first two days and then a shift to daily 100 IU doses for the rest of the LPS.

Several clinics have now switched to an all GnRHa trigger approach and one clinic have as a routine implemented the low-dose hCG daily LPS (i.e. 100 IU daily until a positive pregnancy test) representing the clinical data in routine practice on this approach. The preliminary results of the first almost one hundred patients are presented in table 1. Both groups of patients received COS independent of the LPS provided and have similar characteristics. Final maturation of oocytes was induced by an GnRHa trigger in both groups but the LPS differed; one group received a bolus of hCG of 1.500 IU on the day of OPU plus in some cases an additional bolus of hCG on OPU+5, whereas the other group received 100 IU hCG daily from the day of OPU. It is noticeable that the clinical pregnancy rate is increased by 9% in favour of the daily low-dose hCG group and that the

rate of early pregnancy loss is zero. Obviously these data needs to be taken with caution, this is not a RCT with all the potential bias's it may include, but does warrant further investigations and a proper RCT.

The ultimate goal may be to develop a long acting hCG variant in connection with the GnRHa trigger that can provide a constant low level of hCG in the physiological range throughout the luteal phase, potentially providing a new alternative LPS. This drug remains to be developed, but this analysis demonstrates that new strategies to improve reproductive outcome in IVF still remain to be explored.

Collectively, the present study demonstrates that revisiting the use of hCG for LPS may represent one option to introduce the exogenous progesterone free concept and simultaneously improve reproductive outcome. It is also suggested that gentle low-dose hCG administration for LPS is a strategy that may result in a reduced incidence of OHSS. There are multiple ways of stimulating the CL to produce high levels of progesterone and there are ample opportunities to individualise treatment. Although the administration of low-dose hCG in a clinical setting is still not in routine practice and requires additional instructions and preparation, it will provide better comfort and convenience to the patients, as they can avoid vaginal progesterone pessaries or painful im. injections. It may be envisioned that a long-acting hCG molecule which could provide continuous physiological concentrations of hCG during the luteal phase would be the future solution.

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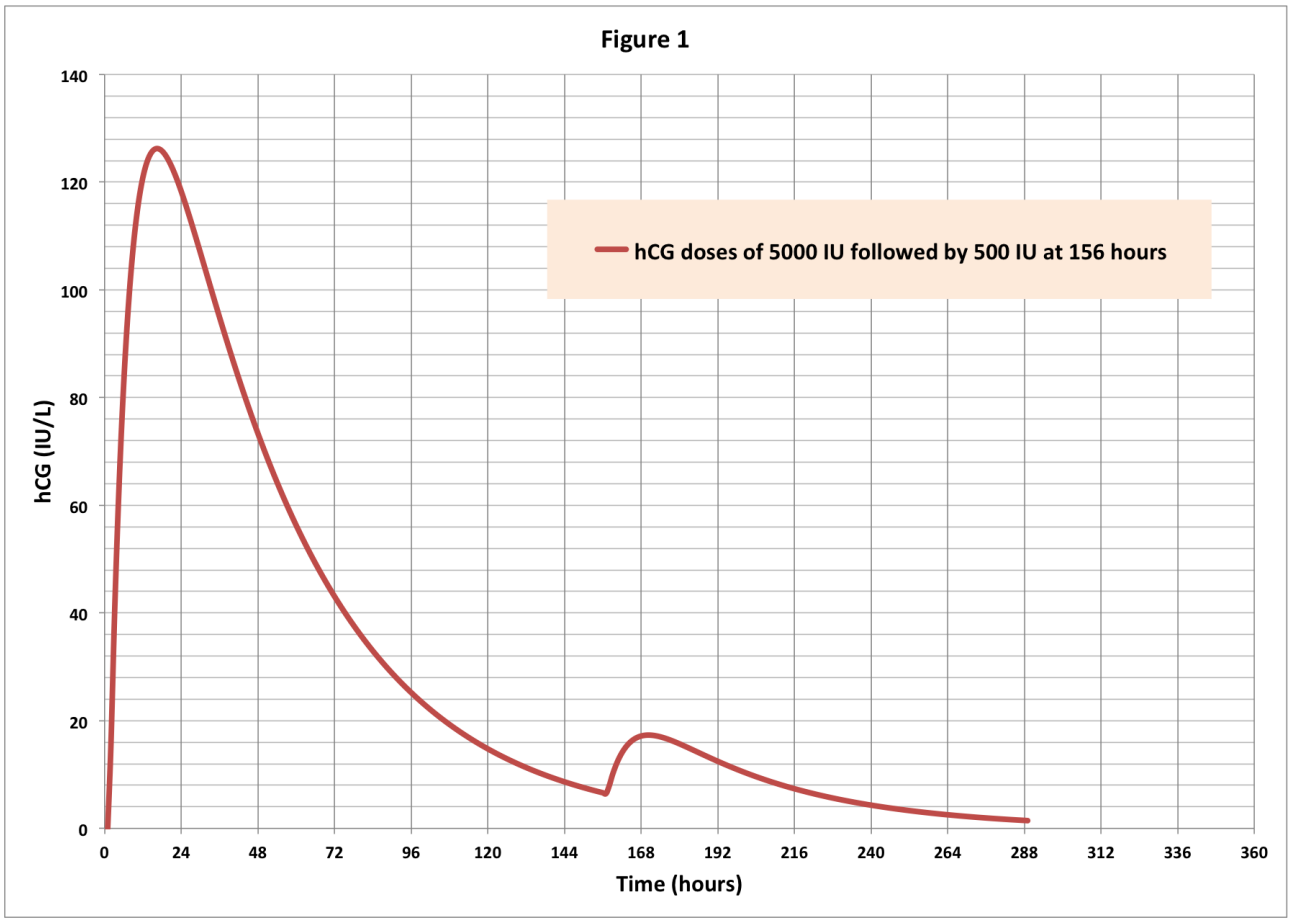
Legend Figure 1.

The graph represents the circulatory concentrations of hCG after exogenous hCG administration of 10.000 IU hCG followed by four administrations of either 1.500 or 2.500 IU of hCG during the luteal phase. Data are calculated based on the information from exogenous administration of 250 µg recombinant hCG (Trinchard-Lugan et.al (2002)), fitted to represent a fit to a pharmacokinetic model with first order absorption and linear elimination including a lag time.

Legend Figure 2.

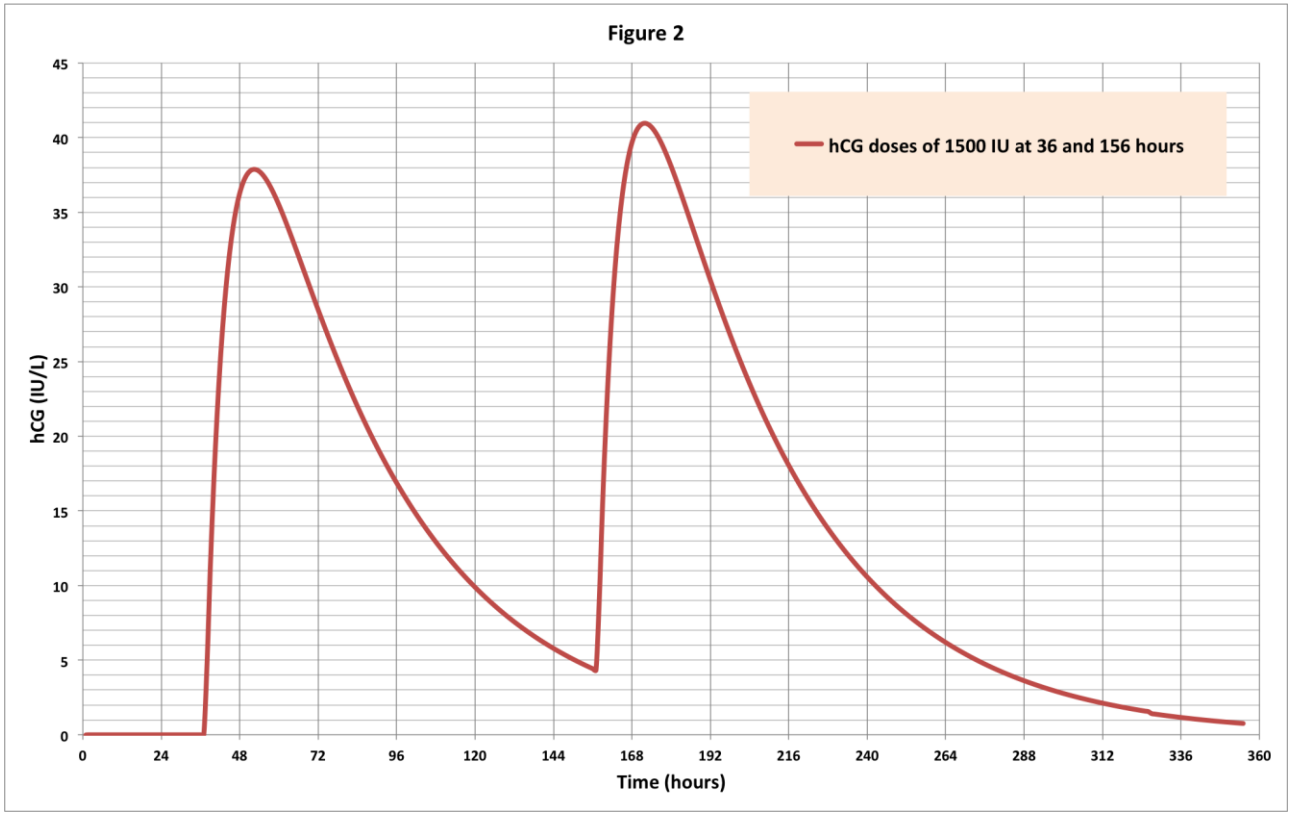
The graph represents the circulatory concentrations of hCG after exogenous hCG administration of 5.000 IU hCG for final maturation of follicles followed by 500 IU hCG on day OPU+5. The calculated concentration of hCG on day OPU+7 is 9.4 IU/L. For data calculation see legend to fig.1.



Legend Figure 3.

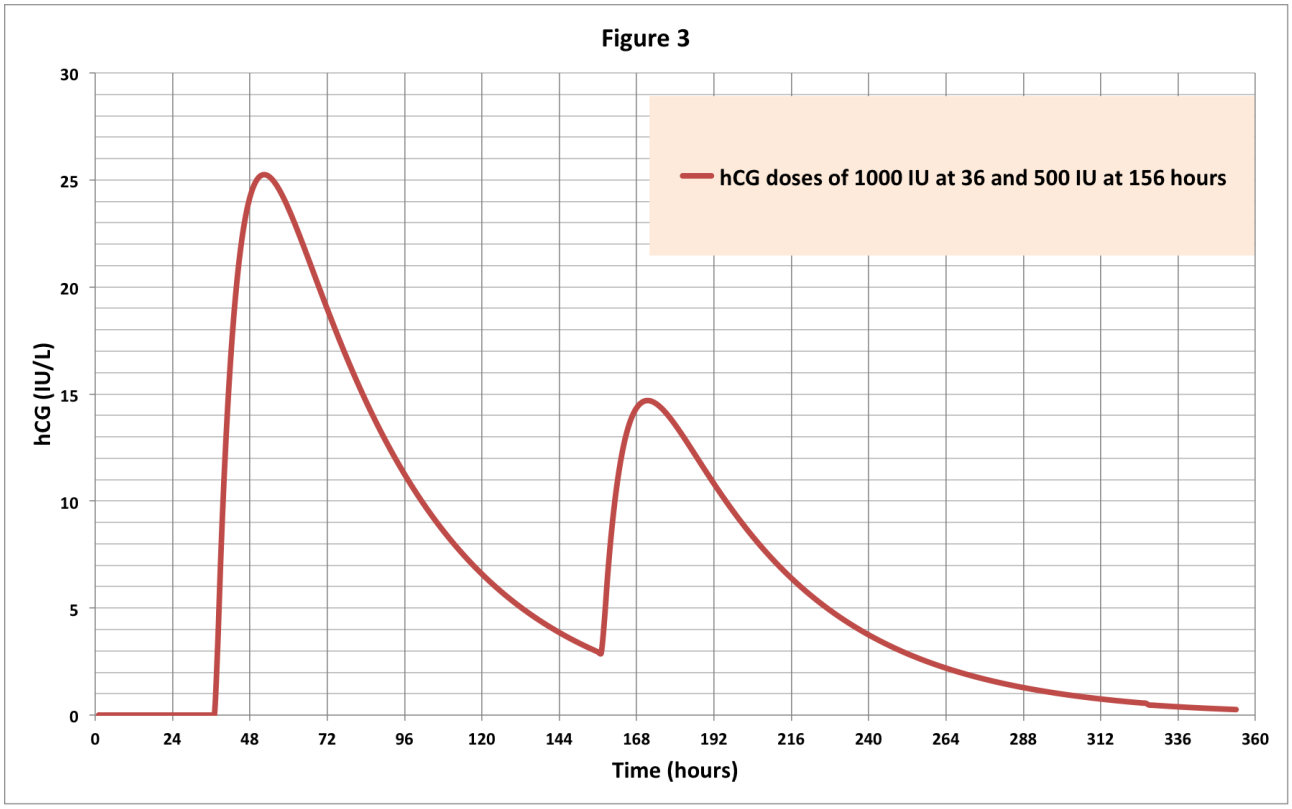
The graph represents the circulatory concentrations of hCG after use of the GnRHa trigger for final maturation of follicles (devoid of hCG activity) followed by administration of 1.500 IU hCG at OPU

and 1.500 IU at day OPU+5. The calculated concentration of hCG on day OPU+7 is 23 IU/L. For data calculation see legend to fig.1.



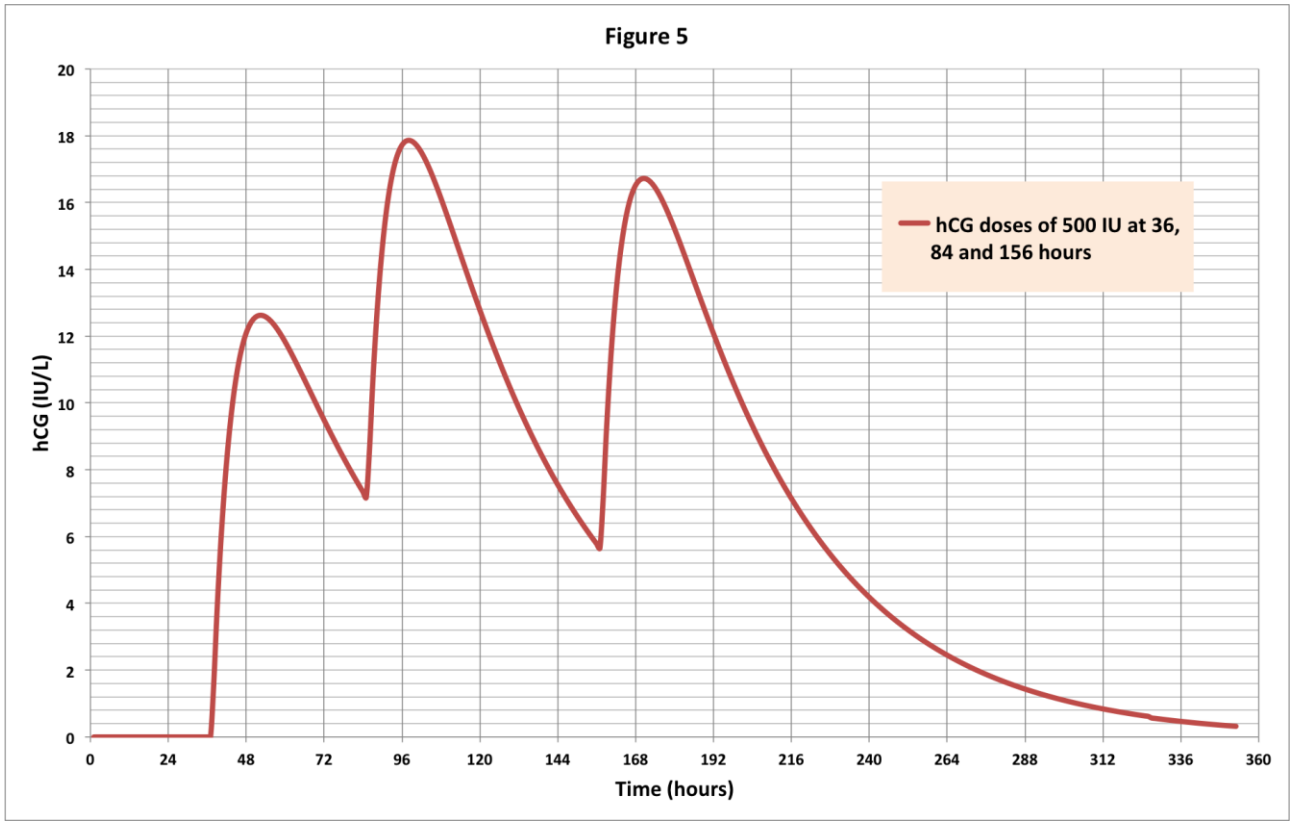
Legend Figure 4.

The graph represents the circulatory concentrations of hCG after use of the GnRHa trigger for final maturation of follicles (devoid of hCG activity) followed by administration of 1.000 IU hCG at OPU and 500 IU at day OPU+5. The calculated concentration of hCG on day OPU+7 is 8.2 IU/L. For data calculation see legend to fig.1.



Legend Figure 5.

The graph represents the circulatory concentrations of hCG after use of the GnRHa trigger for final maturation of follicles (devoid of hCG activity) followed by administration of 500 IU hCG at OPU, 500 IU at OPU+2 plus and 500 IU at day OPU+5. The calculated concentration of hCG on day OPU+7 is 9.1 IU/L. For data calculation see legend to fig.1.



Legend Figure 6.

The graph represents the circulatory concentrations of hCG after use of the GnRHa trigger for final maturation of follicles (devoid of hCG activity) followed by daily administration of either 100, 125 or 150 IU hCG throughout the luteal phase. The calculated concentration of hCG on day OPU+7 is ≈ 6 IU/L, ≈ 8 IU/L, ≈ 9.5 IU/L . For data calculation see legend to fig.1.