The role of the IVF clinician is to make the ART treatment safe, patient-friendly, cost-effective and at the same time offer good and high quality treatment. IVF protocols are a burden for women and are one of the potential reasons why women don’t return for subsequent cycles. Frequent injections may increase stress and also result in high error rates. Simple short treatment regimen with optimal recovery of good quality oocytes results in development of good quality embryos followed by SET in treatment and cryopreservation cycles are a less burden and result in related lesser discontinuation, side effects, treatment cycles in time and are more cost-effective. Development of FSH analogues with longer terminal t1/2 and slower absorption to peak serum levels will increase the efficiency, decrease the side effects and also is easy to administer. This makes it convenient for the patients increasing the compliance. A certain minimum LH concentration is necessary for adequate thecal cell function and subsequent oestradiol synthesis in the granulosa cells. Adjuvant r-HLH gives clinician’s precise control over the dose of LH bioactivity administered to target the therapeutic window. New parenteral, transdermal, inhaled and oral fertility drugs and regimens are currently under research and development with the objective to further simplify treatment for ART.

**KEY WORDS:** Assisted reproductive technology, corifollitropin alfa, gonadotrophins, ovulation induction, stimulation

**INTRODUCTION**

Gonadotrophin therapy is an essential component in the routine management of infertility for both assisted reproductive technology (ART) and non-ART cycles. A lot of research was necessary in order to develop preparations that are safe and effective for clinical use. The history of this process originated with early attempts to extract and purify preparations from animals, human cadavers and human urine, eventually evolving to their production by recombinant DNA technology. The process of evolution to produce recombinant molecules derived from Chinese hamster ovary (CHO) cells has been constantly driven by the need to make gonadotrophin (GT) products safe, pure, and effective not only in treatment but also in ease of administration to the patient. Reliable batch-to-batch consistency is also required for a steady response.

Milestones in gonadotrophin therapy with the first pregnancies reported after its use is seen in Table 1.

The role of the IVF clinician is to make the ART treatment safe, patient-friendly, cost-effective and at the same time offer good and high quality treatment. IVF protocols are a burden for women and are one of the potential reasons why women don’t return for subsequent cycles. Frequent injections may increase stress and also result in high error rates. Simple short treatment regimen with optimal recovery of good quality oocytes results in development of good quality embryos followed by SET in treatment and cryopreservation cycles are a less burden and result in related lesser discontinuation, side effects, treatment cycles in time and are more cost-effective.
development of good quality embryos, followed by SET in treatment and cryopreservation cycles are a less burden and result in related lesser discontinuation, side effects, treatment cycles in time and are more cost-effective.

Initially gonadotropins were produced from the human menopausal urine, which was highly purified, though not totally. The problem of the short supply of high-quality gonadotrophins and purity was solved by the advent of recombinant DNA technology, which permitted the large-scale production of pure recombinant human gonadotrophin preparations with purity of 99% and high in vivo bioactivity.[2-4]

In this review we will discuss on long acting FSH corifollitropin alfa, recombinant LH and FSH and LH receptor agonist and antagonist.

CORIFOLLITROPIN ALFA

Development of FSH analogues with longer terminal t₁/₂ and slower absorption to peak serum levels will increase the efficiency, decrease the side effects and also is easy to administer. This will make it convenient for the patients thus increasing the compliance. Development of corifollitropin alfa is the first step towards a new generation of recombinant gonadotrophins.

DEVELOPMENT OF CORIFOLLITROPIN ALFA

Boime et al., attached the CTP of the hCG b-subunit to the FSH b-subunit using site-directed mutagenesis and gene transfer techniques.[5] They constructed a chimeric gene containing the sequence encoding the CTP of the hCG b-subunit fused to the translated sequence of the human FSH b-subunit. The FSH b-CTP chimera was then transfected with the common glycoprotein a-subunit and expressed in Chinese hamster ovary (CHO) cells.

STRUCTURE OF CORIFOLLITROPIN ALFA (ORG 36286)

Corifollitropin alfa is a hybrid molecule with sustained follicle stimulating activity. It is a recombinant fusion molecule of FSH and the carboxyl terminal peptide (CTP) of hCG b subunit.[6] It is a gonadotrophin with different pharmacokinetic properties but similar pharmacologic features as the available FSH in the market today. Presence of CTP component, which contains four O-linked oligosaccharides gives it a prolonged half-life compared with rFSH.[6] It has a similar in vitro receptor binding and steroidogenic activity compared with wild-type FSH but, had significantly enhanced in vivo activity and plasma half-life with t₁/₂ -65 h, tₘ₉₅ - 25–45 h.[7] It interacts only with the FSH receptor and lacks LH activity.

FSH-CTP is produced by Chinese Hamster Ovary cells. Using site-directed mutagenesis and gene transfer techniques the CTP extension of hCG-β was coupled to the FSH-β unit. It was found that the presence of the CTP sequence did not significantly affect assembly or secretion of the intact dimmer by stable cell lines.[8]

Development of long acting molecules can be done by:
1. Linkage of CTP to recombinant hormones
2. Introducing additional sequences containing potential glycosylation sites at the N-terminus of the FSH a-subunit
3. Fusion with the constant region fragment (Fc) domain of immunoglobulin G1-Two forms of FSH were created[10]
4. By creating a contiguous, single-chain, covalently-bound fusion protein containing the common a-and FSH b-subunits separated by the hCG b-CTP[6,11,12]
The table below gives the comparison between corifollitropin alpha and recombinant FSH [Table 2].

**METHOD OF ADMINISTRATION**

Before the start of ovarian stimulation, pregnancy should be excluded by means of an hCG test, a blood sample taken for hormone assessments, and ultrasound performed to measure and count visible follicles and to rule out the presence of an ovarian cyst.

The treatment cycle was started on menstrual cycle day 2 or 3 as depicted in Figure 1. Stimulation starts with a single s.c. injection of 150 μg (0.5 mL) corifollitropin alfa (NV Organon, The Netherlands). To prevent premature LH surges the GnRH antagonist ganirelix (0.25 mg, Orgalutran/w ganirelix acetate injection, NV Organon, The Netherlands) was administered once daily s.c. starting on stimulation day 5 up to and including the day of hCG. From stimulation day 8 onwards, treatment is continued with a daily s.c. dose of (active) 150–200 IU rFSH up to the day of hCG. Urinary hCG (10 000 IU) or Rec hCG 250 mcg should be administered to induce final oocyte maturation as soon as at least three follicles of 17–18 mm are observed by transvaginal USG.\(^2\)

Corifollitropin alfa, due to its long half-life, one single injection may replace the first 7 injections with conventional gonadotropins during a fertility treatment cycle.

**Trials before corifollitropin was introduced in clinical practice**

The efficacy of corifollitropin alfa has initially been investigated in a small feasibility trial,\(^1\) followed by a larger multicentre dose-finding trial in women undergoing ovarian stimulation for IVF or intracytoplasmic sperm injection (ICSI). The results of the dose-finding trial showed a significant dose–response relationship with respect to the number of cumulus–oocyte–complexes retrieved.\(^1\)

**The feasibility study**

The first RCT was a Phase II FS evaluating the efficacy and safety of corifollitropin alfa in COS-IVF.\(^1\) A total of 98 women were randomized to receive a 120, 180 or 240 μg injection of corifollitropin alfa on cycle day 2 or 3. Women who did not reach hCG criterion on day 8 received 150 IU/day rFSH injections from stimulation day 8 onwards. Women allocated to the control group received 150 IU/day rFSH injections from the start. Daily GnRH antagonist injections were started on the day when the leading follicle reached ≥ 14 mm size. Up to three embryos were transferred on day 3 or 5 after fertilization.

**ENGAGE TRIAL**

In the Engage trial, ongoing pregnancy rates were assessed in 1506 treated patients after one injection of 150 μg corifollitropin alfa during the first 7 days of stimulation and compared with seven daily injections of 200 IU human rFSH using a standard gonadotropin-releasing hormone (GnRH) antagonist protocol in patients from North America and Europe. Ongoing pregnancy rates of 38.9% for the corifollitropin alfa group and 38.1% for the rFSH group were achieved, with an estimated nonsignificant difference of 0.9% (95% confidence interval [CI], −3.9% to +5.7%) in favor of corifollitropin alfa.\(^1\) Equivalent ongoing pregnancy rates by treatment were independent of whether patients underwent IVF or intra-cytoplasmic sperm injection (ICSI), had single or double embryo transfer or embryo transfer on day 3 or 5.

The new treatment option with corifollitropin alfa in a GnRH antagonist protocol is simpler and more convenient than daily rFSH treatment for patients undergoing assisted reproductive technology (ART).\(^1\) However, there could be a loss of flexibility with the corifollitropin alfa treatment option. Flexibility options of clinical importance include: (i) The start day of stimulation, (ii) the option of a 24-h delay in administration of hCG to induce final oocyte maturation, (iii) the option of receiving rFSH on the day of hCG, and (iv) the option of a step-down or fixed-dose of rFSH from day 8.
Four randomized trials involving 2,326 women were included in a recent systematic review. No statistically significant difference was observed in the ongoing pregnancy rate for corifollitropin alfa versus rFSH. There was an evidence of increased ovarian response and risk of OHSS in corifollitropin alfa. Regarding OHSS incidence per woman randomized in four papers reviewed, the number needed to harm (NNT) was reported as 100, with an absolute risk increase of 1% after corifollitropin alfa. This review concluded that in view of its equivalence and safety profile, corifollitropin alfa in combination with daily GnRH antagonist seems to be an alternative for daily rFSH injections in normal responder patients undergoing ovarian stimulation in IVF/ICSI treatment cycles.[17]

This trial concluded that treatment flexibility at the start or completion of ovarian stimulation does not substantially affect clinical outcome in patients treated with corifollitropin alfa or in those treated with daily rFSH for the first 7 days of COS using a GnRH antagonist protocol.

ENSURE TRIAL

Previously, the dose-finding trial of corifollitropin alfa indicated that body weight is a major determinant of response after exposure to corifollitropin alfa and treatment outcome.[14] The Ensure trial was taken up to determine the effect of a single dose of 100 μg corifollitropin alfa to maintain multiple follicular development during the 1st week of stimulation in patients weighing <60 kg. This was a double-blind, double-dummy, randomized, equivalence trial in 396 women weighing 60 kg or less who underwent controlled ovarian stimulation prior to IVF or intracytoplasmic sperm injection (ICSI) were randomized in a 2:1 ratio to a single dose of 100 μg corifollitropin alfa or daily 150 IU recombinant FSH (rFSH) for the first 7 days of stimulation in a gonadotrophin-releasing hormone antagonist protocol. The 2:1 randomization ratio was used to collect more safety information on the investigational product and did not introduce bias due to the double-blind, randomized design of the trial.[18]

The mean ± SD number of oocytes retrieved per started cycle was 13.3 ± 7.3 for corifollitropin alfa versus 10.6 ± 5.9 for rFSH. The estimated treatment difference of +2.5 oocytes (95% CI 1.2–3.9) in favour of corifollitropin alfa (P < 0.001) was well within the predefined equivalence margin. The median (range) duration of stimulation was 9 (6–15) days in both groups. In 32.8% of the patients, one injection of corifollitropin alfa was sufficient to reach the human chorionic gonadotrophin criterion. The incidence of moderate and severe ovarian hyperstimulation syndrome was 3.4% for corifollitropin alfa and 1.6% for rFSH. A dose of 100 μg corifollitropin alfa offers a simplified treatment option for potential normal responder patients with a lower body weight.

A single injection of 100 μg corifollitropin alfa had a safety profile comparable to daily doses of 150 IU rFSH in terms of the incidence and type of reported serious adverse events and adverse events. In line with the higher ovarian response, the incidence of moderate/severe OHSS tended to be higher after treatment with corifollitropin alfa than after treatment with rFSH, although the difference was not statistically significant. Corifollitropin alfa was well tolerated at the site of injection and no drug-related hypersensitivity reactions or anti-corifollitropin alfa antibodies were reported. These findings are consistent with the outcome of previous phase I to III trials.[14,15,19,20]

This trial concluded that a lower dose of corifollitropin alfa (100 μg) offers a simplified treatment option for potential normal-responder patients with a lower body weight (at most 60 kg) undergoing controlled ovarian stimulation prior to IVF or ICSI. Compared with the reference group, treated with a fixed starting dose of 150 IU rFSH, the ovarian response is higher following corifollitropin alfa but well within the predefined equivalence margin where as the duration of stimulation is equally short. One-third of the patients studied had complete multiple follicular development up to three follicles ≥17 mm with a single injection of 100 μg corifollitropin alfa and did not need additional rFSH injections.

The Engage and Ensure trial, which was initiated to predict the effects of a range of single doses of corifollitropin alfa followed by daily rFSH treatment for ovarian stimulation. When taking various variables including age and body weight into account, the dose modelling for stimulation revealed that 100 μg is the most optimal corifollitropin alfa dose in the desired 1 week regimen for women with a body weight up to and including 60 kg and provides an exposure similar to the exposure provided by 150 μg in women weighing more than 60 kg. Equal exposure to those two dosages would also imply equal ovarian response.[21]

TRUST TRIAL

The TRUST trial assessed immunogenicity and safety profile of corifollitropin in detail.[22] Pre- and post-treatment serum samples were obtained and tested for the potential anti-corifollitropin antibodies by a validated highly sensitive assay. Participants of the TRUST study were systematically examined 30 min after each corifollitropin injection for injection site pain, itching, swelling, and redness. No drug-related hypersensitivity reactions were reported.[23] None of the participants had moderate or severe injection site reactions. The safety issues looked at were
immunogenicity, adverse events and incidence of ovarian hyperstimulation syndrome and multiple pregnancies.

**Immunogenicity**

Corifollitropin alfa has a deviated carbohydrate site chains that are foreign to humans and therefore it can be immunogenic and lead to drug-related hypersensitivity. No anti-corifollitropin alfa or anti-CHO antibodies were detected in the participants of this Phase I study. This finding was confirmed in over 1000 women who received corifollitropin in other Phase II and III studies conducted to date.[13,14,18,19,24]

In another trial by Norman R J it was observed that repeated treatment cycles with a single injection of 150 mg corifollitropin alfa can be safely and effectively applied in potential normal responder patients undergoing COS prior to IVF or ICSI, without concerns for immunogenicity.[23]

**Adverse events**

A total of 46.8%, 35.2%, and 31.3% of TRUST study participants reported at least one adverse event after cycles 1, 2, and 3, respectively.[23] Pain associated with oocyte collection procedure, pelvic pain, pelvic discomfort, headache, and antepartum bleeding and mild injection site reactions, formed the majority of adverse events. The incidence of these adverse events in the TRUST study was similar to that in earlier reports. Incidence of severe adverse events was very low. Severe adverse events were reported in 2.5%, 1.3%, and 0.5% in cycles 1, 2, and 3, respectively.[23]

The incidence of serious adverse events was 6.9% and included eight ectopic pregnancies, two ruptured ectopic pregnancies, one heterotopic pregnancy, three missed abortions, and two imminent abortions. The proportion of women and nature of serious adverse events were similar between the corifollitropin and rFSH arms in both the ENGAGE and ENSURE trials.

**Ovarian hyperstimulation syndrome (OHSS)**

We know that corifollitropin injection has sustained FSH levels during the 1st week and the dose adjustment is not possible for that time has raised concerns about OHSS risk. Increased follicular recruitment with corifollitropin injection as reflected by rapidly increasing serum E2 levels and the higher number of 11 mm follicles observed within the first 6 days can justify such concern. OHSS occurred with similar incidence in the corifollitropin and rFSH arms of individual trials (5.4% vs. 8.0% in the FS, 2.6% vs. 2.4% in the DFS, 7.0% vs. 6.3% in ENGAGE, and 6.7% vs. 4.7% in ENSURE), despite the fact that women with polycystic ovary syndrome (PCOS) and women who had a previous history of OHSS had been excluded from all trials.[13-15,18,23]

Similarly, women who had an over response or OHSS in a cycle were discontinued from the TRUST study.[23] Therefore, these observations cannot be extrapolated to women with the highest risk of OHSS.

**Multiple pregnancy**

Number of embryos transferred were similar in both corifollitropin and daily rFSH arms of the reviewed trials. The incidence of multiple pregnancy rates was similar in both groups.

**Outcomes of repeated corifollitropin COS cycles**

Total duration of stimulation, the numbers of COCs retrieved, numbers of embryos generated, good quality embryos, and embryos transferred were similar in the first, second, and third COS cycles of the TRUST trial.[23] Embryo implantation, vital pregnancy and ongoing pregnancy rates seem consistent across fresh embryo transfer cycles. Cumulative ongoing pregnancy rate including spontaneous pregnancies and pregnancies achieved with frozen thawed embryo transfer between stimulated cycles was 61%.

**Use of corifollitropin alfa with GnRh agonist**

There is only one study published on use of corifollitropin alfa with GnRH agonist as all trials were conducted on its use with GnRH antagonist. A single-dose of 100 μg or 150 μg corifollitropin alfa in a long GnRH agonist protocol is able to support multi-follicular development during the 1st week of ovarian stimulation.[15] This study observed that number of follicles, serum E2 and number of oocytes retrieved indicate a relatively high ovarian response. However, further controlled studies are needed to support efficacy and safety of corifollitropin alfa in a long GnRH agonist protocol.

On the basis of phase I, II, III and Engage and Ensure trial following information was obtained about corifollitropin alfa.
**PHARMACOKINETICS**

The results of phase I and phase II trials in pituitary-suppressed volunteers and patients, respectively, show that the mean $t_{1/2}$ of corifollitropin alfa is approximately 65 h for all doses tested between 60 and 240 mg [Figure 2] compared with approximately 35 h for rFSH. Peak levels reached within 36–42 h as compared to 10–12 h for rFSH. Dose normalized (dn) area under the curve (AUC) and dn $C_{max}$ are similar across all doses, indicating that the PK parameters of corifollitropin alfa are dose-proportional over this range. Median $C_{max}$ of corifollitropin alfa is reached between 25 and 45 h after injection. No differences were observed between the PK in volunteer’s pituitary suppressed by oral contraceptives and nonsuppressed patients undergoing ovarian stimulation in a GnRH antagonist protocol. Elimination of corifollitropin alfa is not largely affected by body weight, but exposure is inversely correlated to body weight, exhibiting a linear relationship to both serum clearance and volume of.

In summary, the single-dose PK of corifollitropin alfa is characterized by a slow absorption resulting in peak levels within 2 days after injection. Thereafter, serum corifollitropin alfa levels decrease steadily, though the FSH activity may be retained above the FSH threshold for an entire week if the administered dose of corifollitropin alfa is sufficiently high.

**EFFICACY**

The pharmacokinetics profile of corifollitropin alfa after a single injection implies the highest FSH activity [Figure 3] during the first 2 days of stimulation, followed by decreasing FSH activity until treatment with daily FSH is started. Single injection induces and sustains multi-follicular development during the 1st week of stimulation and is effective in stimulation of multi-follicular growth for IVF but less suitable for induction of monofollicular growth and therefore lU.

The maximal serum concentration of collifollitropin increased with the doses injected. Maximum serum FSH-CTP concentrations were 0.42, 0.66, 1.49 and 3.27 ng/ml after administration of 15, 30, 60 or 120 μg respectively. When statistical analysis was performed, no statistically significant differences between doses were found for any of the (dose normalized) pharmacokinetic parameters. Thus absorption of FSH-CTP was much slower and the elimination half-life was twice as long as that of rFSH. Initial trials studies a dose range of 15–120 μg and the elimination half-life (ranging from 60 to 75 h) was dose independent.

Dose-finding Trial of corifollitropin alfa was initiated in 2008 where 60, 120 and 180 μg were studied. Single dose of corifollitropin alfa sustains follicular growth for an entire week in all the 3 groups but the number of follicles that are recruited vary with dose. High cancellation rate in the 60 μg dose group (44%) indicated that dose was too low to support the first 7 days of ovarian stimulation.

**FOLLICULAR GROWTH**

Transvaginal ultrasonography results showed that single FSH-CTP administration induced follicular growth in almost all subjects. Follicles with a diameter > 8 mm were only observed in the 60 and 120 μg group. The maximum diameter of follicles in the 60 μg group was between 8.0 and 9.9 mm and between 14.0 and 15.9 mm in the 120 μg group. In the higher dose groups, large cohorts of follicles were recruited. When comparing the ultrasonography results of this study with results from previous work, the effect of a single administration of 120 μg FSH-CTP on follicular growth appears to be slightly reduced compared with 7 days administration of 150 or 225 IU rFSH. This implies that, to obtain an effect similar to that of seven daily rFSH injections, the dose of FSH-CTP should be further increased. Thus, FSH levels would remain above the threshold level for follicular stimulation during a longer time period, and probably weekly administration would be sufficient. Statistically significant dose-related increase in number of follicles 11 or 15 mm on day 8 of stimulation and hCG administration was noted. There was also a statistically significant increase in number of oocytes retrieved over this dose range.

**HORMONE LEVELS**

Serum FSH immunoreactivity increased rapidly until stimulation day 3 (postinjection day 2) in recipients...
of corifollitropin alfa. The peak level depended on the corifollitropin alfa dose administered and exceeded the peak levels achieved in recipients of daily rFSH in both ENGAGE and ENSURE trials. There were no significant differences between serum FSH levels in corifollitropin alfa and rFSH groups from day 8 onward.[15,18]

Serum E2 levels tended to increase faster following corifollitropin alfa injection than after daily rFSH injections.[13‑15,18] This is consistent with the higher serum FSH levels and higher number of follicles ≥11 mm observed in the corifollitropin alfa arms during the initial days of stimulation. On stimulation day 8, serum E2 levels had a dose–response relationship with corifollitropin alfa dose.[14] However, serum E2 levels on the day of hCG injection was not significantly different between corifollitropin alfa and rFSH groups.[13‑15]

Serum levels of inhibin-B, which is produced by granulosa cells and is an early marker of follicular growth increased dose-dependently after FSH-CTP administration.[19]

Inhibin B levels were similar between corifollitropin alfa and daily rFSH regimens. The only exception was the sharp decline after day 6 in the 60 μg corifollitropin alfa arm of the dose finding study.[14] This observation is consistent with a lower number of growing follicles in recipients of the 60 μg dose and further confirms that this dose is inadequate for COS.

LH levels declined rapidly after corifollitropin alfa injection in all groups. However, women who received the 240 μg dose had increasing LH levels from the 3rd day after corifollitropin alfa injection.[13] LH levels also declined following commencement of GnRH antagonist injections in these women. With the flexible GnRH antagonist protocol used in the feasibility study, LH rises before the start of the GnRH antagonist injections were observed in 16.2% who received corifollitropin alfa as compared to 8.3% who had daily rFSH injections.[13] Although the incidence of premature LH surges before starting GnRH antagonist was not significantly different between the groups, this has led to starting GnRH antagonist injections on the 5th day of stimulation in the following trials. Despite the early start of GnRH antagonist, a premature LH rise of 10 IU/L was detected by day 5 in 5.1% women in the corifollitropin alfa arms of the dose finding study.[14] 13 None of the women in the rFSH arm had a premature LH rise before GnRH antagonist injection in the same study. Notably, three women in the corifollitropin alfa arms and two in the rFSH arm had a premature LH rise after GnRH antagonist injections.[14]

The incidence of premature LH rises were 7% versus 2.1% (P < 0.01) and 5.2% versus 3.9% (P = 0.57) in the corifollitropin alfa and rFSH arms of the ENGAGE and ENSURE trials, respectively.[15,18] The pregnancy rates for women with premature LH rises were not significantly different between corifollitropin alfa (45.3%) and rFSH (31.3%) groups of the ENGAGE trial.[15]

The higher incidence of premature LH rises observed in the corifollitropin alfa groups seems to result from the higher FSH exposure during the early follicular phase. Similar observation has been reported when women with high ovarian reserve were given higher starting dose of daily rFSH injections in earlier trials evaluating GnRHant.[27‑29]

Progesterone levels remained low throughout the stimulation period in all corifollitropin alfa groups and were not significantly different than women who received daily FSH injections. Likewise, luteal phase hormone profiles were not significantly different between corifollitropin alfa and rFSH groups.[13‑15,18]

EMBRYOLOGY LABORATORY PARAMETERS

Fertilization rate was similar across different doses of corifollitropin alfa, as well as between corifollitropin alfa and rFSH groups in all trials.[13‑15,18] Although the number of embryos available tended to be higher in corifollitropin alfa arms (especially in the 120 μg arm) of the feasibility study, the numbers of good quality embryos were similar across all groups including the rFSH group.[13] Women in the 120 and 180 μg arms of the dose finding study had significantly higher numbers of total embryos and good quality embryos than women in the rFSH arm probably due to higher numbers of COCs retrieved in these groups. The numbers of embryos available and good quality embryos were similar in corifollitropin alfa and rFSH arms of the ENGAGE and the ENSURE trials.[15,18] The mean number (standard deviation) of embryos cryopreserved in the corifollitropin alfa and the rFSH groups were 4.3 (3.6) versus 3.9 (2.7) and 2.0 (3.0) versus 1.7 (2.6) in the ENGAGE and the ENSURE trials respectively.[15,18]

RESULTS WITH CORIFOLLITROPIN ALFA

Incidence of viable pregnancy and ongoing pregnancy rates per cycle and per transfer was similar in the four arms of the DFS.[14] Ongoing pregnancy rate per fresh cycle ranged between 16% and 24% in the corifollitropin alfa arms of the FS and the DFS.[13,14] Cumulative ongoing pregnancy rate including transfer of frozen thawed embryos obtained from the index cycle within a year was only reported in the DFS, and figures were 18% in the 60 μg, 27% in the 120 μg, 24% in the 240 μg corifollitropin alfa groups, and 20% in the...
daily rFSH group. Early pregnancy losses during the first 10 weeks were also comparable in the two groups. Percentage of patients who discontinued treatment either for poor or excessive response was also similar in the two groups.

Thus, initial trials concluded that the optimum dose of corifollitropin alfa to sustain follicular development for 1 week was > 60 μg and lower than 180 μg. Later Organon’s initiated the ENGAGE and ENSURE trials for corifollitropin alfa for its therapeutic indication of COH.

The ENGAGE and Ensure trials, which used the corifollitropin alfa dose adjusted for body weight had results different from the FS and DFS. The ENGAGE trial, which used the corifollitropin alfa dose of 150 μg for women > 60 kg and a higher starting dose of daily rFSH injections reported an ongoing pregnancy rate per cycle 38.9% which was similar to 38.1% rFSH groups (P = 0.7). Ongoing pregnancy rates per cycle achieved in the ENSURE trial, which used 100 μg of corifollitropin alfa in women < 60 kg was 25.4% as against 34.4% in the rFSH group (P = 0.06). When the results from the two RCTs using bodyweight-adjusted gonadotrophin dosages are combined, ongoing pregnancy rates were not significantly different between corifollitropin and daily rFSH groups (odds ratio 0.95, 95% confidence interval 0.79–1.15.

**Safety**

No antibodies against FSH-CTP or CHO-derived proteins were detected. The FSH-CTP preparation was well tolerated. No serious adverse events (SAE) were observed and none of the subjects discontinued due to adverse events (AE). There were no clinically relevant adverse events and no relevant changes in laboratory parameters.

Trust Trial focuses on safety of repeated treatments with corifollitropin alfa. All tested doses were safe and well-tolerated, no anti-corifollitropin alfa antibodies were detected and OHSS with hospital admission occurred in 2–3%.

Disadvantages of corifollitropin alfa:

1. Dose cannot be reduced to obtain milder stimulation
2. Serum FSH levels decline after stimulation day 3 (Cmax) onwards
3. Dose reduction during 1st week of stimulation cannot be made in case of hyper response
4. Less suitable in cases with known risk of hyper-response− PCOS, previous OHSS
5. PR and LBR not yet confirmed to be comparable with daily Rec FSH
6. Ovarian response induced may decrease with the patient’s age and ovarian reserve.

The ovarian response to corifollitropin alpha is dependent on clinically established predictors such as baseline FSH, antral follicle count (AFC) and age. There is a general trend towards a higher ovarian response with an increasing AFC and the number of oocytes per attempt decreased with increasing baseline FSH and age. Even if the risk of ovarian hyperstimulation syndrome following corifollitropin alpha is very similar to the rate reported in literature for young women undergoing IVF, the risk of overstimulation may be reduced by avoiding maximal ovarian stimulation in women anticipated to be hyperresponders. High basal anti-Mullerian hormone and/or AFC can identify women with enhanced functional ovarian reserve at risk of overstimulation, and the risk is even higher if maximally stimulated with corifollitropin alpha or high dose of daily recombinant FSH.

**RECOMBINANT LH**

A certain minimum LH concentration is necessary for adequate thecal cell function and subsequent oestriadiol synthesis in the granulosa cells. The consequent rise in oestriadiol concentration is essential for endometrial proliferation and corpus luteum formation in anticipation of a fertilized oocyte, implantation and embryo development in pregnancy. It therefore stands to reason that LH concentrations that are too low will increase the likelihood of unsuccessful implantation or early pregnancy loss. LH and FSH have complementary functions in ensuring optimal oocyte maturation and ovulation. Growing follicles become increasingly sensitive to, and ultimately dependent on, the presence of LH for their development in the mid and late follicular phase. Women undergoing ART use protocols with gonadotrophin-releasing hormone analogues, which result in reduced concentrations of LH and FSH. We know that FSH has a definitive role in folliculogenesis but there is no published consensus on the need for exogenous LH, which can be used either as recombinant human LH (r-HLH), human menopausal gonadotrophin (hMG) or human chorionic gonadotrophin (hCG) in a very small dose. Some ART practitioners advocate add-back LH to the mid-follicular phase in ovarian stimulation cycles, while others deem add-back LH to be unnecessary, justifying that the small amounts of LH present after down-regulation are sufficient to sustain theca and granulosa cell stimulation. If right patient is not chosen for administration of Rec LH and is given indiscriminately to all patients early overexposure of LH in ovarian stimulation can result in premature follicle luteinization of small follicles and follicular atresia leading either to cycle cancellation due to follicle maturation arrest or to poor-quality oocytes, all of which translates into severely compromised outcomes.
Supplementation of LH may benefit selected women with LH deficiency and suboptimal ovarian response during ART as measured by clinical end-points such as oestradiol concentrations and follicular development.

Human menopausal gonadotrophin (hMG) or human chorionic gonadotropin (hCG) and are subject to wide variation in LH quantity and bioactivity. It was observed that adjuvant r-HPH gives clinicians’ precise control over the dose of LH bioactivity administered to target the therapeutic window. Recombinant-HPH is associated with high purity, precision of dosing and consistency. When administered by subcutaneous injection, r-HPH has a terminal half-life of 24 h.[33] It is structurally and functionally analogous to endogenous human LH.

Possible mechanisms for the improved implantation and clinical pregnancy may be due to increased oocyte competence or improved endometrial receptivity. Cycles with recombinant LH supplementation have also shown lower levels of cumulous cell apoptosis than FSH-only cycles, possibly indicating improved oocyte quality in LH-supplemented cycles.[34] As patients age, there is an increase in early follicular phase FSH but not LH[35] and it is possible that the administration of LH restores the follicular milieu of the developing follicle in older ART patients.[36]

Apart from hypo gonadotropic hypogonadism the use of r-HPH is recommended in women with poor response in a previous cycle or suboptimal follicular progression in a current cycle by days 6–8 of stimulation. r-HPH should also be considered in women at risk of suboptimal response, specifically age >35 years. Other risk markers that suggest the need for LH supplementation, which include baseline/day 6 serum LH and anti-Müllerian hormone concentrations, antral follicle count and LH polymorphisms require further research and verification. For measurement of LH response adequacy, the monitoring of follicular progression, oestriadiol concentrations and endometrial thickness is recommended.[32]

DOSE AND TIMING OF INITIATION OF R-HPH

The dose of r-HPH in hypo-hypo patients for r-HPH is 75 IU combined with 150 IU r-HFSH, that is, a 2:1 ratio of FSH to LH. In patients undergoing ART with prevention of LH surge using GnRH analogues, on the combination of r-HPH and r-HFSH in suboptimal responders used r-HPH doses of 75–150 IU daily combined with r-HFSH doses of 300–375 IU.

In a study that compared either 75 IU or 150 IU r-HPH with r-HFSH (follitropin a or follitropin b) in suboptimal responders with r-HFSH alone in normal responders, significantly more oocytes were retrieved from the 150 IU r-HPH plus r-HFSH group.[37]

Regarding the timing of initiation of r-HPH in ovarian stimulation, in some protocols patients start on r-HPH from day 1 of stimulation and for others patients start on days 6–8. Currently, there is no evidence supporting either day 1 or days 6–8 for starting r-HPH. However, in theory there may be a benefit to starting patients on day 1 if a clinician wants to maximize the benefit of increased ovarian androgen production. LH supplementation from day 1 may increase circulating androgen concentrations and it has been demonstrated that increased androgens in combination with FSH can act synergistically to promote FSH receptor mRNA expression, follicular development and steroidogenesis.[38]

In normogonadotrophic women undergoing long GnRH agonist protocol IVF cycles and treated with r-HFSH were 5 times more likely to suffer early pregnancy loss if LH serum concentrations on stimulation day 8 were below 0.5 IU/l (P < 0.005).[39] This was further supported by a recent two-treatment arm RCT[22] that compared r-HFSH versus r-HFSH combined with r-HPH, in a long agonist assisted reproduction technology cohort with day 6 LH concentrations <0.5 IU/L. There were no differences between the groups in the number of oocytes retrieved (6.37 ± 2.67 vs. 7.32 ± 1.99, respectively); however a significantly higher number of mature oocytes were obtained from the group receiving r-HPH (136 vs. 93, P < 0.05) and fertilized oocytes (92% vs. 69%, P < 0.001). Clinical pregnancy rate was 5% for r-HFSH alone compared with 22% with r-HFSH plus r-HPH (P < 0.05).

In another RCT in patients with a suboptimal response to stimulation with a long GnRH agonist stimulation protocol that compared adding higher doses of r-HFSH versus adding r-HPH or HMG, those given r-HPH (n = 54) had higher live-birth rates (40.7%) than those given HMG (18%). This study also concluded that r-HPH group also had higher implantation rates.[40]

In a study published by Kolibianakis et al. concluded that there was no significant difference was observed in the probability of live birth with or without rLH addition to FSH with odds ratio, OR= 0.92, 95% CI = 0.65–1.31andal P = 0.65.[41] No significant difference was observed in the gonadotrophin consumption, duration of stimulation, estradiol and progesterone levels on the day of HCG, number of cumulus complexes retrieved and fertilization rate. Thus available evidence does not support the hypothesis that the addition of recombinant LH increases the live birth rate in patients treated with FSH and GnRH analogues for IVF.
Hugues et al. evaluated the addition of recombinant LH in WHO group II anovulatory women over-responding to FSH treatment to reduce the number of developing follicles. They concluded that in patients over-responding to FSH during ovulation induction, doses of r–HLH up to 30 mg/day are well tolerated in the late follicular phase and appear to increase the proportion of patients developing a single dominant follicle. [42]

Papanikolaou et al. published a study on role of r–HLH for luteal phase support role in an attempt to reverse the poor reproductive outcome previously noticed after GnRH-agonist triggering of final oocyte maturation for IVF. The study group received 0.2 mg of triptorelin (Ipsen, Boulogne Billancourt, France) for ovulation triggering, standard P luteal support, as mentioned previously, plus six doses every other day of 300 IU recombinant LH starting on the day of oocyte retrieval up to day 10 after oocyte retrieval. The control group received 250 mcg of recombinant hCG for ovulation triggering and standard luteal support, as mentioned previously, plus six doses every other day of 300 IU recombinant LH starting on the day of oocyte retrieval up to day 10 after oocyte retrieval. The total number of oocytes retrieval were higher in the rFSH + rLH group but the total number of MII oocytes is approximately the same. The reduction of the amount of FSH used in the hMG group also led to lower cost of the IVF cycle and of the babies born. There was an increase in the OHSS risk in the Pergoveris group, which explains the statistically significant difference in the cancelled patient rate. [43]

Future studies are needed to define the minimal requested dose of recombinant LH to achieve appropriate circulating LH levels that ensure implantation (also unknown) and to determine the intervals at which we should repeat the injected recombinant LH and up to which day.

**RECOMBINANT FSH PLUS RECOMBINANT LH (PERGOVERIS)**

Treatment with hMG or with rFSH plus rLH could achieve the same results in term of pregnancy rate, implantation rate, and embryo quality, but statistical difference in oocytes quality was seen, with a better quality in the hMG group. The total number of oocytes retrieval were higher in the rFSH + rLH group but the total number of MII oocytes is approximately the same. The reduction of the amount of FSH used in the hMG group also led to lower cost of the IVF cycle and of the babies born. There was an increase in the OHSS risk in the Pergoveris group, which explains the statistically significant difference in the cancelled patient rate. [44]

**IN RESEARCH**

**Oral and pulmonary delivery of FSH–Fc fusion proteins via neonatal Fc receptor-mediated transcytosis**

Heterodimer FSH–Fc is also significantly more active than single chain FSH–Fc. FSH–Fc fusion proteins have increased stability in blood and improved bioactivity in vivo, and that heterodimer FSH–Fc is more active in rats and monkeys than single chain FSH–Fc. Data on its use in rats and monkeys suggest that Fc fusion proteins offer the potential for oral and pulmonary delivery of FSH. The half-life of heterodimer FSH–Fc in cynomolgus monkeys is 182–219 h. This is significantly longer than the half-life of rFSH of, 24 h in humans [43] and in nonhuman primates. [16, 47] Thus an obvious advantage of using heterodimer FSH–Fc in infertility treatments is the potential for a greatly reduced dosing frequency. In addition, pulmonary or oral delivery of FSH–Fc fusion proteins using endogenous FcRn expressed in epithelial cells of the lung and intestine could significantly improve tolerability of current infertility treatments.

**Low molecular weight gonadotrophins**

Consistent with other therapeutic areas, novel drug development in the infertility field is likely to concentrate on less invasive delivery methods, such as the use of long-acting compounds or different routes of administration that may include transdermal, inhaled or oral agents. On the horizon is the development of orally active, low molecular weight gonadotrophins, for which a first proof-of-concept study has been reported in female volunteers. [48]

**Induction of ovulation by a potent, orally active, low molecular weight agonist (Org 43553) of the luteinizing hormone receptor**

Org 43553 is the first LMW LH-R mimetic with demonstrated in vivo efficacy upon oral administration and could therefore replace subcutaneously administered hCG. It is a thienopyrimidine compound class. It is a pure synthetic molecule lacking the variability between batches as observed for proteins of urinary or recombinant origin. It is completely protein free, thus totally excluding the minimal current risk for diseases like Creutzfeld Jacob’s, originating from recombinant gonadotrophin production in the presence of bovine serum in the culture media. [49]

On the basis of the pharmacokinetic profile of Org 43553 in rat, the anticipated human half-life of Org 43553 was calculated with a method described by Bachman et al. [50] Using this method, the anticipated human half-life of Org 43553 was found to be between 15 and 30 h after a single-dose oral treatment. In a first human exposure study, the human elimination half-life of Org 43553 after oral administration was proven to vary between 30 and 47 h. [13] which is substantially and remarkably shorter than the elimination half-life of hCG (48–96 h in humans). The shorter half-life of Org 43553 in humans compared with hCG may have a reduced risk for OHSS. Thus, it can be developed as a safe oral alternative to the current injectable LH/hCG preparations.
for clinical use to induce ovulation or oocyte maturation for both \textit{in vivo} and IVF therapy. In addition, the compound can also be developed for male indications such as hypogonadism.

Small molecule agonists and antagonists for the LH and FSH receptors.

Luteinising hormone (LH) and follicle-stimulating hormone (FSH) play a critical role in human reproduction. LH and FSH are secreted from the pituitary and act on their respective G-protein-coupled receptors (GPCRs), LHR and FSHR, in the gonads to either promote follicular growth and differentiation in women or to stimulate the proper progression of spermatogenesis in men. LH and FSH are currently used in the clinic for the treatment of infertility. Small molecule agonists of LHR and FSHR have the potential to become oral therapeutics for infertility treatment, whereas small molecule antagonists of LHR and FSHR may find utility in oral contraception. Advances in molecular biology, high-throughput screening (HTS) and combinatorial chemistry have made significant contributions to the recent discovery of a variety of small molecule LHR and FSHR agonists and antagonists, some of which have shown highly promising efficacy in animal models of fertility control.

CONCLUSION

Corifollitropin alfa is the first long-acting hybrid molecule with sustained follicle-stimulating activity developed for the induction of multi-follicular growth along with GnRH antagonist co-treatment for IVF. Corifollitropin alfa is a synthetic recombinant follicle-stimulating hormone (rFSH) molecule containing a hybrid beta subunit, which provides a plasma half-life of $\sim 65$ h while maintaining its pharmacodynamic activity. Corifollitropin alfa has a slower absorption rate and two-fold longer plasma $t_1/2$ than recombinant FSH, while maintaining the same pharmacodynamic activity. This allows a single injection of corifollitropin alfa to effectively replace the first seven daily injections of rFSH in COS cycles for anticipated normo-responder women undergoing \textit{in vitro} fertilization. Stimulation can be continued with daily FSH injections if the need arises. In women who received corifollitropin alfa, one-third of them did not require additional gonadotropin injections and reached human chorionic gonadotropin criterion on day 8. The optimal corifollitropin dose has been calculated to be 100 $\mu$g for women with a body weight $\leq 60$ kg and 150 $\mu$g for women with a body weight $\geq 60$ kg, respectively. Combination of corifollitropin with daily gonadotropin-releasing hormone antagonist injections starting on stimulation day 5 seems to yield similar or significantly higher numbers of oocytes and good quality embryos, as well as similar ongoing pregnancy rates compared with women stimulated with daily rFSH injections. Stimulation characteristics, embryology, and clinical outcomes seem consistent with repeated corifollitropin-stimulated assisted reproductive technologies cycles. Multiple pregnancy or ovarian hyperstimulation syndrome rates with corifollitropin were not increased over daily FSH regimen. Further research is needed to determine whether corifollitropin can be used for women with anticipated poor or hyper-response. The corifollitropin alfa molecule does not seem to be immunogenic and does not induce neutralizing antibody formation. Drug hypersensitivity and injection-site reactions are not increased. Incidence and nature of adverse events and serious adverse events are similar to daily FSH injections. Current trials do not provide information regarding use of corifollitropin alfa in anticipated hyper- and poor responders to gonadotropin stimulation. Although corifollitropin alfa is unlikely to be teratogenic, but there are no human data regarding congenital anomaly rate following corifollitropin stimulation available in the literature. Using corifollitropin alfa in combination with a fixed daily GnRH antagonist will further simplify treatment and may reduce the treatment burden of IVF for patients. But it remains to be objectively demonstrated whether switching to corifollitropin decreases anxiety and distress associated with ART.

Although current treatments are increasingly successful, treatment-related burden may be reduced by less intervention. Corifollitropin alpha is a highly effective gonadotrophin, which maintains multi-follicular growth for a week. The advantages of its administration include ease of use of the drug, making the treatment more patient friendly, resulting in a lower level of distress for the patient. At the same time, the pregnancy rate resulting from its use in IVF/ICSI cycles is similar to that found when daily recombinant FSH is administered.

Corifollitropin alfa has a advantage that it reduces the number of injections that the patient needs to take. However, its use requires the correct prediction of response especially expected high responders for whom the drug is contraindicated. The safe clinical use of corifollitropin alpha is only secondary to a consistent in label use and to a good capability of the clinician to predict the potential ovarian response in a given patient. If the patient is a hyper-responder predicted by AFC and AMH values, the patient is at a higher risk to develop OHSS and the use of the corifollitropin alpha should be avoided. Conversely, if the patient is a low or a normal responder and not at risk of developing the OHSS, its use is a really valid treatment option in IVF.

For prevention of premature LH surge the multiple low-dose GnRH antagonist protocol should be considered for each patient as compared to the long GnRH agonist protocol which is time consuming and stressful for the patient.
The strongest predictive factor for need of exogenous LH in assisted reproduction technology is a prior poor or suboptimal response to ovarian stimulation. Another important group who benefit from adjuvant r-HLH in addition to r-HFSH are women who exhibit suboptimal ovarian response during ovarian stimulation as characterized by:

a. No follicle > 10 mm by days 6–8
b. Low oestradiol (<180 pg/ml) by day 6
c. Poor progression or slowing of follicle growth, with previously 1–2 mm progression per day slowing to <2 mm in 3 days.

In women undergoing ART, who are >35 years there is increasing evidence that age is an important marker of deficient LH bioactivity and benefit from r-HLH supplementation. The meta-analysis published by Hill et al.[51] concluded that patients of advanced reproductive age had higher implantation and clinical pregnancy in recombinant LH plus recombinant FSH protocols compared with recombinant FSH-only protocols but did not influence the oocyte or MII yield.

New parenteral, transdermal, inhaled and oral fertility drugs and regimens are currently under research and development with the objective to further simplify treatment for ART.

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