

Presence of LH in gonadotropins associated with higher IVF pregnancy rates when basal serum LH is increased

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Summary

Purpose: To determine if pregnancy rates following in vitro fertilization-embryo transfer (IVF-ET) correlate with the presence or not of luteinizing hormone (LH) in the gonadotropins used for stimulation. Furthermore to see if the early follicular phase serum LH level affects pregnancy outcome according to the type of gonadotropins used.

Methods: The type of gonadotropins were prescribed randomly according to finances and convenience. Serum LH was obtained on day 2 or 3 of the menstrual cycle.

Results: When LH was > the median, significantly higher pregnancy rates were obtained in those treated with the follicle stimulating hormone (FSH)/human menopausal gonadotropin combination. When LH was \leq the median, significantly more oocytes were retrieved with FSH exclusively. No confounding variables were found to explain the data.

Conclusions: Considering concerns of published studies that LH may have a toxic effect on pregnancy outcome, and if LH is suppressed too low, gonadotropins with exclusive FSH may not stimulate sufficient oocytes, the results were opposite to expectations.

Key words: FSH; Human menopausal gonadotropins; Median LH.

Introduction

Several studies have demonstrated an adverse affect of elevated concentrations of follicular phase serum luteinizing hormone (LH) on subsequent conception rates and outcomes in women with polycystic ovarian syndrome (PCOS) [1-6]. Too much LH during the time of follicular development and in the periovulatory phase may have detrimental effects on fertilization, cleavage and embryo quality [7, 8]. Thus one of the theoretical advantages of a gonadotropin preparation devoid of most of the LH, in contrast to human menopausal gonadotropins (hMG), where equal concentrations of follicle stimulating hormone (FSH) and LH are present, may be to circumvent the adverse effect of LH [9]. Though LH is needed to stimulate the theca cells in synergy with inhibin to produce androgens, only low levels of LH are needed for follicular maturation [10].

However, in contrast to FSH where there is a slower clearance, LH is usually completely eliminated 24 hours after hMG injection [11]. Some studies have found that the administration of hMG to patients with polycystic ovarian syndrome does not result in a significant increase in LH concentration [12, 13].

A meta-analysis by Daya *et al.* [14] of randomized trials of FSH versus hMG used for controlled ovarian hyperstimulation (COH) with or without gonadotropin releasing hormone agonists (GnRHa) [15-20] concluded that in in vitro fertilization (IVF) cycles, the use of FSH is exclusively associated with a significantly higher clinical pregnancy rate than hMG [14]. A randomized con-

trolled trial by Daya *et al.* [21] found a significantly higher fertilization rate with FSH vs hMG; however, though there was a trend for higher pregnancy rates with FSH, there was not a significant difference. Another study by Jansen *et al.* [9], also found higher pregnancy and implantation rates with recombinant FSH vs hMG. Thus, whether the adjunct of LH is necessary, somewhat beneficial, or detrimental has been an ongoing matter of debate [22].

All these studies compared all FSH vs all hMG. No studies to date have compared FSH and hMG vs FSH alone. Since hMG is less expensive than FSH, we received approval from the research and ethics committee of the Cooper Center for In Vitro Fertilization to randomize women receiving the luteal phase leuprolide acetate-gonadotropin COH regimen into treatment arms of either FSH alone vs FSH and hMG combined in IVF cycles, according to whether their insurance paid for most of the medication (where they received all FSH) or whether the medications were paid out of pocket (where they received 50% FSH, 50% hMG). The study would not only determine if adding some LH to stimulation regimens would lower pregnancy rates, but would determine if any protocol was superior according to the serum LH levels obtained in the early follicular phase.

Materials and Methods

Consecutive IVF cycles using the luteal phase leuprolide acetate-gonadotropin protocol in 1998 were evaluated according to whether they took all FSH (Fertinex, or Gonal-F - Serono Inc., or Follistim - Organon Inc.) or a mixture of FSH and hMG (Pergonal, Serono Inc.; Humegon, Organon Inc.; Repronex, Ferring Inc.).

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All patients were started on 0.75 mg leuprolide acetate one week after ovulation and this was continued for at least ten days until the serum estradiol (E2) was < 50 pg/ml and the serum progesterone (P) < 1.5 ng/mL. At this time the leuprolide acetate dosage was decreased to 0.5 mg S.C. daily and 300 IU gonadotropins in two divided doses were used. Human chorionic gonadotropins (hCG) (10,000 units) were given IM when at least two follicles were attained with an average diameter of 20 mm. Oocyte retrieval was performed 36 hours thereafter. The decision on which gonadotropins to use was based strictly on finances, i.e., those with prescription coverage were given all FSH therapy and those without were given FSH/hMG to save them some money since hMG was available at a much lower price.

Serum LH was measured at baseline (day 3) before leuprolide acetate, after ten days of the GnRHa, after five days of gonadotropins, and on the day of hCG. Follicle stimulating hormone was measured at baseline only.

The data was evaluated according to whether the patient's baseline LH levels were at or lower than our median baseline LH for our IVF center of 4 mIU/ml or above this level. Parameters assessed total ampules of gonadotropins used, mature oocytes retrieved, percent of fertilization of mature oocytes, number of embryos available per patient, and clinical and viable pregnancy rates, implantation rates, and spontaneous abortion rates. Also, outcome from frozen embryo transfers (ET) were assessed.

Embryo transfers were performed three days after oocyte retrieval. Twice as many embryos as the patient wanted to be transferred would be allowed to develop to day 3 and the best half were selected for fresh ET, and the deselected embryos were cryopreserved using a simplified freezing protocol using 2 propanediol as the cryoprotectant [23]. The remainder of the embryos were cryopreserved at the 2 pronuclear (2PN) stage. Assisted embryo hatching was performed prior to transfer using acidic Tyrode's solution on fresh [24] and frozen [25] embryos. For frozen ETs also, twice as many embryos as intended for transfer were thawed and the deselected ones were refrozen [26]. When there was a choice between 2PN and multi-cell embryos for culturing to day 3, the 2PN ones were chosen first.

Patients were stratified by their baseline LH levels. Group 1 consisted of patients with baseline LH at or below the group median of 4.0 mIU/mL; group 2 consisted of patients with baseline levels above the median. Within each group, the outcome of ovarian stimulation and embryo transfer were compared by stimulation used. Chi-square analysis and independent t-test were used as appropriate. A p value of .05 was used.

Results

A comparison of serum LH levels by ovarian stimulation and baseline LH levels can be seen in Table 1. The LH levels were similar within LH groups 1 and 2, except for the women in the higher LH group (group 2) taking FSH and hMG, where the LH was higher on day 5 of gonadotropins than the women taking all FSH. Serum baseline FSH was also significantly higher in group 2 women taking mixed gonadotropins compared to group 2 women taking all FSH, but there were no differences in serum baseline FSH in group 1 women.

A comparison of ovarian response by ovarian stimulation and baseline LH levels can be seen in Table 2. There were no differences seen in the total amount of medica-

tion used. The mean number of mature oocytes retrieved was 19.1 in the patients using all FSH in the lower LH group compared to only 12.2 in the group using FSH/hMG ($p < .05$). As expected the group with more oocytes had more embryos (15.2 vs 10.2) ($p < .05$). No differences were found in the type of FSH product used either (urinary versus recombinant).

For group 1 patients with lower baseline serum LH, there were no differences seen in the clinical pregnancy rate, implantation rate or spontaneous abortion rate or even the pregnancy rate including the first frozen ET (if the fresh transfer was deferred) whether the COH regimen was all FSH or FSH/hMG as seen in Table 3. However, all of these parameters, except spontaneous abortion rate were significantly higher ($p < .05$) in the women in the higher LH group taking FSH/hMG (Table 3).

Discussion

We have previously determined after evaluating 500 IVF cycles that the median baseline LH for patients seen at our IVF center is 4 mIU/ml. The median for this study was also 4 mIU/ml. We thought that if one analyzes the data according to whether the patient starts with an LH at the median or below, or above the median, perhaps the LH: FSH ratio of the dosage used for COH might have different effects on outcome.

Indeed significant differences were seen in two major categories: total number of mature oocytes generated and pregnancy and implantation rates. However, these differences were opposite to what might have been expected.

Edelstein *et al.* [27] showed that a reduction in ovarian response could be demonstrated in previous high responders with hMG by using all FSH. This makes one wonder if the same principle would hold for poor responders, i.e., less response with FSH vs hMG, which in this case would be detrimental. However, our own study found no difference in follicle number in GnRHa-gonadotropin cycles with hMG or FSH [15]. One non-IVF study in patients with polycystic ovaries, and thus increased LH levels, showed a greater tendency to hyperstimulate with all FSH [28]. However, the present study found that group 1 patients taking all FSH made significantly more follicles than those using the mixed protocol. Though no significant differences in serum LH from baseline to day of hCG were found according to type of gonadotropin stimulation in group 1, we looked to see if there may be a fortuitous trend that could explain these data. However, the women receiving exclusive FSH therapy had a baseline LH of 2.2 vs 3.0 mIU/ml for patients receiving mixed gonadotropins and serum LH and was also lower on day 10 of leuprolide acetate (2.7 vs 3.6 mIU/ml) and after five days of gonadotropins (1.9 vs 7.6 mIU/ml). Thus, these data cannot be explained by subtle higher LH levels (albeit not significantly higher) in the women receiving all FSH. A greater potential for more oocytes could be explained if there was a fortuitous selection of women receiving all FSH with lower baseline FSH levels, but these values were almost identical for those

Table 1. — Comparison of serum LH levels by ovarian stimulation and baseline LH levels (data presented as mean \pm standard deviation).

| Ovarian stimulation | Baseline LH \leq 4.0 mIU/ml | | Baseline LH $>$ 4.0 mIU/ml | |
|------------------------------|-------------------------------|----------------|----------------------------|----------------|
| | FSH/LH (n=30) | All FSH (n=23) | FSH/LH (n=25) | All FSH (n=18) |
| Age | 32.8 \pm 3.6 | 33.1 \pm 3.4 | 31.9 \pm 3.5 | 33.0 \pm 4.5 |
| Baseline LH | 3.0 \pm 1.0 | 2.2 \pm 1.2 | 7.2 \pm 2.6 | 6.5 \pm 2.3 |
| Baseline FSH | 5.6 \pm 2.3 | 5.0 \pm 2.2 | 7.1 \pm 1.9* | 5.5 \pm 1.7* |
| LH on day 10 of lupron | 3.6 \pm 1.7 | 2.7 \pm 1.5 | 4.3 \pm 3.1 | 5.0 \pm 2.2 |
| LH on day 5 of gonadotropins | 2.6 \pm 1.9 | 1.9 \pm 1.4 | 4.6 \pm 3.2* | 2.5 \pm 1.6* |
| LH on day of hCG | 2.1 \pm 1.8 | 2.6 \pm 2.3 | 4.6 \pm 3.7 | 2.8 \pm 1.6 |

* p < .05 comparing stimulation protocols within LH groups.

Table 2. — Comparison of ovarian response by ovarian stimulation and baseline LH levels (data presented as mean \pm standard deviation).

| Ovarian stimulation | Baseline LH \leq 4.0 mIU/ml | | Baseline LH $>$ 4.0 mIU/ml | |
|-----------------------------------|-------------------------------|------------------|----------------------------|-----------------|
| | FSH/LH (n=30) | All FSH (n=23) | FSH/LH (n=25) | All FSH (n=18) |
| Total amps of medication | 43.0 \pm 13.2 | 46.0 \pm 14.7 | 43.5 \pm 16.1 | 44.6 \pm 14.0 |
| Mature eggs retrieved | 12.2 \pm 8.2* | 19.1 \pm 11.6* | 14.6 \pm 6.7 | 14.7 \pm 8.6 |
| % fertilization (mature) | 69.8 \pm 25.1 | 69.4 \pm 13.9 | 62.3 \pm 21.5 | 62.5 \pm 29.1 |
| No. embryos available per patient | 10.2 \pm 7.7* | 15.2 \pm 8.5* | 11.1 \pm 5.2 | 9.3 \pm 5.5 |

* p < .05 comparing stimulation protocols within LH groups.

receiving exclusive FSH stimulation vs the women receiving mixed gonadotropins (5.0 mIU/ml vs 5.6 mIU/ml).

If LH has some toxic effect on pregnancy outcome, then one might expect that patients starting with the highest baseline LH may be more prone to compounding that problem by giving them even more LH in their stimulation protocol [1-4, 7, 8]. Surprisingly, group 2 women receiving mixed gonadotropins actually had a significantly better pregnancy outcome than group 2 women receiving all FSH. These results cannot be explained on fortuitous selection of women receiving mixed gonadotropins with a lower baseline FSH level since, actually, the serum baseline FSH turned out to be significantly higher in this group. Thus, if anything, there would have been a bias against this group.

Furthermore, one cannot explain higher pregnancy rates in group 2 receiving mixed gonadotropins by fortuitously selecting those women who had lower serum

baseline LH values than those receiving all FSH, because there was a trend for higher baseline LH levels in the women receiving mixed gonadotropins, and even a significantly higher LH level in the group receiving FSH/hMG after five days of gonadotropins. A study by Out *et al.* [29], found higher pregnancy rates with recombinant FSH than with urinary FSH when used for IVF. However, there was not any subgroup that fortuitously took more recombinant FSH than urinary FSH products that could influence the data [29].

It is a well known fact that patients with polycystic ovarian syndrome have a greater likelihood to hyperstimulate after gonadotropin stimulation [30]. Since characteristically these patients have higher baseline serum LH levels, many thought that the higher serum LH level played a role in the hyperstimulation process [31, 32]. It was reasoned that patients with polycystic ovarian syndrome might be less inclined to hyperstimulate if LH was removed from the preparation. However, in evaluating urinary FSH (Metrodin, Serono, Inc.) for stimulation vs hMG (Pergonal, Serono Inc.) it was found that FSH was far more likely than hMG to cause the ovarian hyperstimulation syndrome [28]. Though we previously reported a trend for more canceled cycles for inadequate stimulation for IVF in patients taking FSH vs hMG [15], this was at a time when a higher dosage of leuprolide acetate was used (1 mg for 10 days as described in the original protocol by Meldrum *et al.*) [33]. When we dropped the dosage of leuprolide acetate, we found that treating with all FSH was more likely to cause more stimulation of follicles [34]. This previous study did not evaluate the data according to the LH. The results cannot be interpreted that perhaps it is just patients with polycystic ovary tendencies that are the ones more likely to produce more oocytes with exclusive FSH therapy since the women responding the best had the lowest baseline LH levels of all four subgroups and therefore they were the least likely to include patients with polycystic ovaries.

Overall, the group with the lower baseline LH had a clinical pregnancy rate of 48.7% (20/41) vs 45.1% (p=NS) for the group with higher baseline LH levels. Thus, these data with patients undergoing IVF are consistent with conclusions of one of our previous studies on patients not receiving assisted reproductive technology, but in the cases where progesterone supplementation was

Table 3. — Comparison of IVF outcome by ovarian stimulation and baseline LH levels (data presented as mean \pm standard deviation).

| Ovarian stimulation | Baseline LH \leq 4.0 mIU/ml | | Baseline LH $>$ 4.0 mIU/ml | |
|---|-------------------------------|----------------|----------------------------|----------------|
| | FSH/LH (n=30) | All FSH (n=23) | FSH/LH (n=25) | All FSH (n=18) |
| Fresh transfer | 25 | 16 | 15 | 16 |
| Deferred transfer | 5 | 7 | 10 | 2 |
| Risk of OHSS | 5 (16.7%) | 7 (30.4%) | 10 (40.0%) | 2 (11.1%) |
| No. of embryos transferred | 3.0 \pm .8 | 3.2 \pm .4 | 3.3 \pm .5 | 3.2 \pm 1.0 |
| Clinical pregnancy rate/fresh transfer | 48.0 (12/25) | 50.0% (8/16) | 66.7% (10/15)* | 25.0% (4/16)* |
| Implantation rate/fresh transfer | 25.0% (19/76) | 27.4% (14/51) | 30.0% (15/50)* | 11.5% (6/52)* |
| Spontaneous abortion rate | 0.0% | 25% (2/8) | 0.0% | 0.0% |
| Pregnancy rate adjusted for frozen ETs for deferred transfers | 43.3% (13/30) | 47.8% (11/23) | 56.0% (14/25)* | 27.8% (5/18)* |

* p < .05 comparing stimulation protocols within LH groups.

given in the luteal phase, the presence of higher serum LH did not lower pregnancy rates [35].

The meta-analysis by Daya *et al.* [14] and the studies used to form their conclusions [15-20] convinced us to no longer use hMG by itself to save money on medication if it would result in the need for more potential IVF cycles, which in the long run, would cost the patient even more. Since these earlier publications, other studies have also compared all hMG to all FSH and have come to similar conclusions that FSH alone results in higher pregnancy rates than when hMG is used for COH [9, 29, 36-38]. We thus attempted to see if we could reduce medication expenses to a lesser degree but hopefully without jeopardizing success following IVF-ET by using 50% hMG and 50% FSH.

Studies evaluating comparisons of FSH stimulation versus mixture of FSH and preparations with some LH content have been hard to find. One study by Mercan *et al.* [39] found that the use of FSH alone produced better quality oocytes than FSH/hMG combined. Another study comparing recombinant FSH to recombinant FSH and 75 IU of recombinant LH found a trend for higher clinical pregnancy rates per transfer with recombinant FSH alone (68.8% vs 45.5%) [40]. Our study would have found no difference in outcome by FSH alone vs FSH/hMG if we had not evaluated the data according to the serum LH levels. Recently another study, in contrast to previous ones favoring all FSH stimulation, found no difference in pregnancy rates with IVF whether stimulation was with rFSH or hMG [41]. To our knowledge, the study presented here is the first one to compare the efficacy of FSH stimulation versus FSH/hMG according to the serum LH level. Our study is not the only one to suggest some benefit of having LH in the stimulation protocol, at least under certain circumstances, e.g., increased baseline serum LH. Another recent study concluded that using a 50% concentration of LH in the gonadotropins, i.e., hMG alone (Humegon, Organon Inc.) resulted in higher implantation rates compared to products with no LH [42].

These data are certainly provocative and need to be corroborated by other centers before the recommendation is made to evaluate early follicular phase LH levels and add hMG to the stimulation regimen if the baseline LH is higher than the normal baseline median for a given IVF center. A mechanism to explain these findings (which may be opposite as to what might have been predicted) remains to be elucidated.

From a practical standpoint, these results suggest that a given IVF center should determine their own baseline median serum LH level and consider treating with FSH exclusively if the LH level is at the median or below vs using some hMG in the stimulation protocol if the LH is higher than the median. We do not believe that our randomization process based on patients' insurance coverage might have biased the outcome. Theoretically, older, more financially secure patients may have better insurance or more money to afford the more expensive, but more convenient rFSH that can all be given S.C. However, Table 1 shows no differences in age in those

taking hMG/rFSH or all rFSH. There was no difference in our monitoring techniques in these two groups or any attempt to use less ampules of medication. Table 2 clearly shows no differences in the number of 75 IU ampules of gonadotropins used. Nevertheless, we hope that this study will generate a larger, perhaps, multi-center prospective study evaluating whether these conclusions based on early follicular serum LH levels are, in fact, valid. Our study is consistent with the five conclusions reached by Levy *et al.* [43] concerning the role of LH in ovarian stimulation. However, we can modify their conclusions by stating that when one evaluates the data according to median LH, the only potentially adverse effect of LH in the gonadotropins may be a decrease in the number of oocytes retrieved in those with low endogenous baseline LH; but actually for some patients that may be an advantage if there is fear of ovarian hyperstimulation syndrome. Furthermore, we can state that there is actually some benefit in the presence of LH to increase pregnancy and implantation rates in those with higher baseline endogenous LH.

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