The impact of LH-containing gonadotropins on diploidy rates in preimplantation embryos: long protocol stimulation

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BACKGROUND: The aim of this study was to evaluate the effect of ovarian stimulation with LH-containing gonadotropins (human menopausal gonadotropin, hMG), on ploidy of human cleavage-stage embryos. METHODS: A total of 104 women, at ages 27–43 years, undergoing one cycle of controlled ovarian hyperstimulation for IVF in combination with preimplantation genetic diagnosis, were eligible for enrollment in this retrospective, controlled cohort study. Ovarian stimulation included down-regulation with long agonist and stimulation with either recombinant FSH or hMG. Since the ploidy of embryos changes with female age, patients were matched for age and dosage of the respective gonadotropin. RESULTS: Despite similar numbers of chromosomally normal embryos in both groups, women undergoing hMG stimulation demonstrated significantly higher percentages of diploid embryos than did the FSH-stimulated patients (69.8 versus 45.3%; P < 0.01). CONCLUSIONS: Long protocol LH-containing ovarian stimulation improves embryonic ploidy in comparison to pure FSH stimulation. This observation may explain higher IVF pregnancy rates, reported for hMG stimulation in some studies.

Keywords: aneuploidy; preimplantation genetic diagnosis; human menopausal gonadotropin (hMG); LH; rFSH

Introduction

The synergistic relationships of FSH and LH are essential for oocyte development and maturation (Speroff and Kase, 1999). The need for exogenous LH administration during controlled ovarian hyperstimulation (COH) has, nevertheless, remained controversial (Chappel and Howles, 1991; Fleming et al., 1998; Balasch et al., 2001). Those in favor of LH supplementation argue that pituitary desensitization with GnRH agonists or antagonists results in such profound LH suppression that proper oocyte maturation does not take place (Chappel and Howles, 1991; Fleming et al., 1998; Balasch et al., 2001; Lisi et al., 2005). Opponents of that view, however, claim that even marginal concentrations of endogenous LH are sufficient for appropriate oocyte development (Andersen et al., 2006; Barrenetxea et al., 2007).

A recent Cochrane review suggested better clinical pregnancy rates of borderline significance with hMG utilization (Van Wely et al., 2003b). Those data are in accordance with results obtained during the MERIT-study, a large, prospective randomized trial that compared pregnancy rates after long agonist stimulation with either hMG or recombinant FSH (rFSH). It demonstrated significantly higher numbers of top-quality embryos and a trend toward improved pregnancy chances after stimulation with hMG (Andersen et al., 2006). A more recent study, although statistically marginal as well, suggested a possible beneficial effect of co-treatment with LH in respect to pregnancy loss and poor responders (Mochtar et al., 2007).

Although LH is of particular importance during follicular development, its most significant function is probably in the resumption of meiosis (Hsieh et al., 2007). Faultless completion of meiosis I and II is crucial for chromosomal integrity and, hence, oocyte diploidy (Verlinsky and Kuliev, 1996). If meiosis—a process extremely sensitive to disruption—were to be influenced by the presence, or lack, of appropriate LH concentrations, LH supplementation in the course of fertility treatment could, indeed, influence diploidy rates in human cleavage-stage embryos. This study, therefore, evaluated the impact of LH-containing gonadotropins on the chromosomal status of preimplantation embryos during IVF.
Materials and Methods

The study involved 104 women at ages 27–43 years, who underwent one IVF cycle in combination with their first cycle of preimplantation genetic diagnosis (PGD) between January 2004 and June 2005. All IVF cycles were performed at The Institute for Reproductive Medicine and Science, Saint Barnabas (IRMS), Livingston, NJ. PGD analyses were exclusively undertaken at Reprogenetics LLC, West Orange, NJ.

To avoid biases, oocyte donors were excluded from analysis. Infertility patients were eligible for enrollment if they demonstrated evidence of normal, age-appropriate ovarian function, defined as baseline (Day 2/3) FSH levels of up to 10.0 mU/ml and/or an oocyte yield of at least three oocytes after age-appropriate stimulation (Surrey et al., 1998). Women (or their partners) diagnosed as transplantation carriers, or with a history of repeated IVF failure, defined as three or more failed IVF cycles, repeated pregnancy loss, defined as a history of three miscarriages or more, a previous aneuploid conception or moderate to severe male factor infertility were also excluded from analysis.

In order to preclude additional selection biases, only selected PGD cycle indications were considered for analysis. Thus, in older women an acceptable indication for PGD was advanced reproductive age, defined as age 35 and above, while in younger women only prior IVF failure and a desire to minimize embryo transfer numbers were considered as acceptable criteria for inclusion.

Linear regression analyses of the final study population demonstrated no impact of indication for fertility treatment, fertilization method (IVF or ICSI) or, after correction for age, indication for PGD on ploidy, but did demonstrate significant statistical correlations between diploidy and age (P < 0.02) and gonadotropin type as well as dosage (P < 0.05). hMG or rFSH products of different companies yielded comparable results in all investigated parameters. Patients were, therefore, grouped according to gonadotropin use (FSH versus hMG), and both cohorts were matched for female age and gonadotropin dosage. This was done by matching consecutive hMG cycles with FSH cycles, performed during the same time period. Matching allowed for maximal divergence of 150 IU in total gonadotropin usage, and up to one year in age. The person performing the matching was blinded to outcome data.

For both groups, the following parameters were investigated: age, indications for IVF, peak estradiol (E2) levels, days of stimulation, amount (total ampules) and type(s) of gonadotropins used, number of oocytes retrieved, embryo numbers, embryo development (i.e. fragmentation and mean embryonic cell numbers) and, finally, ploidy. All patients underwent standardized long agonist stimulation (Prapas et al., 2005). Pituitary down-regulation with leuprolide acetate was initiated during the midluteal phase of the preceding cycle (Lupron, TAP Pharmaceutical Products Inc., Lake Forrest, IL, USA). Gonadotropin treatment was initiated if baseline E2-values were below 50 pg/ml, and in the absence of follicular structures larger than 10 mm. In all cycles, gonadotropin therapy was initiated with a daily dose of 100–300 IU of either rFSH (Gonal F, Serono, Rockland, MA, USA; Follistim, Organon, Roseland, NJ) or hMG (Repronex and Menopur, Ferring, Suffern, NY) and adjusted according to follicular response. Cycle monitoring, oocyte retrieval, fertilization and embryo transfer were performed in routine fashion, as previously reported (Chen et al., 2005). The decision regarding the number of embryos transferred was based on the total number of available diploid embryos and on the patient’s age and personal preference. Clinical pregnancy was defined as the presence of a gestational sac. The choice of gonadotropin composition (i.e. hMG or rFSH) was left to the physician. Participating physicians did not report specific patient criteria for selection of stimulation protocols. This alone, however, does not preclude physician biases in the selection of such protocols. Since patients were statistically similar in all parameters known to potentially influence the choice of gonadotropin stimulation (i.e. age, number of previous IVF attempts, indication for fertility treatment, baseline hormone parameters), it is reasonable to assume that whatever biases in protocol selection by individual physicians may have been present initially, when compounded, they did not affect the finally selected study groups.

Embryos were placed in a calcium–magnesium-free biopsy medium to loosen blastomere attachments. Acidified Tyrode’s solution was used for zona drilling and consecutive cleavage-stage embryo biopsy on Day 3 after fertilization. Biopsied single cell-blastomeres were then transferred into hypotonic solution and fixed on slides, following a protocol to minimize signal overlap and loss of micronuclei (Velilla et al., 2002).

PGD analysis was generally performed by fluorescence in-situ hybridization, using probes specific for chromosomes X, Y, 13, 15, 16, 17, 18, 21 and 22. As reported previously, hybridization cycle one included probes for chromosomes 13, 16, 18, 21 and 22, (Multi- vysion PB, Vysis, Downer’s Grove, IL, USA), followed by a second round of hybridization with probes for chromosomes 15, 17, X and Y. Due to changing laboratory policies, a slightly smaller number of chromosomes were analyzed in a few cases. Such cases were equally distributed among both groups.

Embryos diagnosed as diploid, contained two distinct signals for each of the chromosomal pairs tested. Polyploidy was diagnosed in the presence of three or more signals for each chromosome, whereas blastomeres with a single copy of each autosome and just one sex chromosome were classified as haploid. Aneuploidy was consistent with an abnormal number of signals for one or two chromosomes. Embryos with abnormal copy numbers for three or more chromosomes, and not fitting in any previously mentioned classification, were considered as complex abnormal (Munne et al., 1998). In some cases, a third round of hybridization, using probes with different locus-binding capacities, was performed to confirm diagnostic results (Colls et al., 2004). Cases that required a third round of hybridization were equally distributed among both groups.

The data analysis of patient baseline characteristics, stimulation, embryology and PGD testing was based on computer-generated databases at IRMS and Reprogenetics. The embryology and PGD data were validated at least once after initial entry by a second technician, showing an error rate of data entry of only 0.3%. Validation of follicular stimulation data was established using a manual review of the medical database and notes.

The influences of female age, total number of IVF attempts, indications for fertility treatment, amount and type of gonadotropins used, number of oocytes retrieved, embryo numbers and absolute number and percentage of diploid embryos were investigated for a total of 104 patients.

P-values <0.05 were considered statistically significant. Quantitative variables are summarized by their range, mean and standard deviation, while qualitative variables are summarized by frequency tables.

Institutional Review Board (IRB) approval for retrospective data analyses was obtained from the IRB at IRMS. At the time of treatment start at IRMS, all patients were informed that their cycle data might be used for research purposes and signed appropriate informed consent forms.

Results

Patient baseline and cycle characteristics are summarized in Table I. Both groups (long agonist stimulation cycles using
FSH and long agonist cycles using hMG) were of comparable mean ages (35.0 ± 3.8 versus 35.9 ± 3.7 years) and presented with similar baseline hormone patterns (FSH, 5.5 ± 2.0 versus 5.5 ± 1.7 mIU/ml; E2, 31.0 ± 17.0 versus 25.9 ± 9.1 pg/ml).

Despite comparable total amounts of gonadotropins used (FSH, 1877.2 ± 660.8 versus hMG, 1722.5 ± 687.7 IU), women undergoing FSH treatment produced significantly higher peak E2 levels (2946.1 ± 1081.8 versus 1945.3 ± 903.6 pg/ml; P < 0.01), and showed trends toward higher oocyte yield (23.7 ± 11.7 versus 20.3 ± 8.4, P < 0.3) and fertilized zygotes (13.8 ± 5.8 versus 11.9 ± 5.6), which, however, did not reach significance.

Both groups presented embryos of comparable quality (mean cell number per Day 3 embryo: FSH, 6.0 ± 1.2 versus hMG, 6.2 ± 1.0; mean fragmentation, FSH, 15.8 ± 14.8 versus hMG, 16.4 ± 8.4). Embryos which did not undergo developmental arrest were available for biopsy. The mean number of embryos analyzed was greater in the rFSH group than in the hMG group (FSH, 8.1 ± 5.0 versus hMG, 5.3 ± 4.4). Although both groups produced comparable numbers of diploid embryos (FSH, 3.1 ± 2.1 versus hMG, 3.3 ± 2.5), women who underwent stimulation with rFSH produced significantly more aneuploid and complex abnormal embryos than women undergoing hMG stimulation (rFSH: aneuploid embryos: 2.2 ± 2.4; complex abnormal embryos: 2.9 ± 2.8; hMG: aneuploid embryos: 0.9 ± 1.2; complex abnormal embryos: 1.2 ± 1.6, P < 0.01). Therefore the rate of diploidy (calculated on a per patient basis) was significantly higher in women undergoing long-protocol stimulation with hMG (FSH, 45.3 ± 26.5 versus hMG, 69.8 ± 26.7%, P < 0.01) (Fig. 1).

When the impact of baseline and cycle characteristics on diploidy rates was evaluated by linear multi-regression analysis, the composition of gonadotropins used (FSH versus hMG) significantly influenced diploidy rates (P < 0.02) and the number of previous IVF attempts and peak E2 levels were significantly associated with the total number of diploid embryos in the two matched cohorts (P < 0.05).

A comparison of clinical pregnancy rates revealed significantly, although borderline, higher pregnancy rates per cycle started after utilization of hMG (rFSH: 44% versus hMG: 63%, P = 0.05), while there was only a trend toward higher clinical pregnancy rates when assessed per embryo transfer (rFSH: 54 versus hMG: 69%). Among these clinical pregnancies, a miscarriage rate of 9% was observed in rFSH cycles, while no miscarriage occurred after stimulation with hMG. Therefore, HMG administration resulted in significantly higher rates of ongoing pregnancies per cycle started (rFSH: 40 versus hMG: 63%, P < 0.02) and per embryo transfer (rFSH: 44 versus hMG: 63%, P = 0.049). Since a mean number of 2.5 embryos were transferred in rFSH cycles, whereas a mean number of 2.8 embryos were transferred in hMG cycles, implantation rates could be calculated for both groups. Implantation rates per embryo transfer reflected pregnancy rates in their respective trends, though the difference failed to reach statistical significance (rFSH: 26.8 versus hMG: 31.6%).

**Discussion**

Our results point toward a beneficial effect of LH-containing stimulation on diploidy rates in preimplantation embryos and possibly, on pregnancy rates. Since diploid embryos are more likely to implant (Munne et al., 2005), higher percentages of diploid embryos will ultimately lead to higher pregnancy chances. These data provide a possible explanation for the results presented by Lisi et al. (2005) and Andersen et al. (2006), who reported higher pregnancy rates in women who receive LH-containing stimulation, rather than pure FSH. They also concur with the 'two cells, two gonadotropins
The likely beneficial effect of hMG stimulation in poor responders, as pointed out by the Cochrane working group, agonist stimulation, using hMG, in comparison to FSH. The finding of comparable numbers of diploid embryos in both groups, but higher diploidy rates in hMG cycles, may offer a potential explanation for some remaining controversies in regards to the impact of LH-containing stimulation on embryo development and pregnancy rates (Balasch et al., 2001; van Wely et al., 2003a).

The following model could explain this in more detail. Hypothetically, two patients of the same age, with comparable infertility history and ovarian function, are both undergoing a cycle of long agonist stimulation for IVF without PGD. The first patient receives FSH, whereas the second is treated with hMG. The first produces 12, and the second 10 embryos. Embryo numbers (and quality) are thus similar; both women are also of similar age and show comparable ovarian function. Similar implantation and pregnancy potentials can, therefore, be assumed for both. Let us, furthermore, assume that both women produced six diploid embryos. The first patient, thus, had six diploid and six aneuploid embryos, whereas the second, of course, will end up with only four aneuploid embryos. Consequently, in the absence of PGD, the first woman will have a lower chance of having two diploid embryos transferred than the second patient, who, in this example, received hMG.

This observation may explain why the likely benefits of exogenous LH supplementation are not seen in all women. They, indeed, may be difficult to detect in studies that do not include appropriately selected patient populations. For example, in patients with low endogenous LH levels (Jia et al., 1985; Balasch et al., 2001; Lindsey et al., 2002), in poor responders and with repeated pregnancy loss (Mochtar et al., 2007), the benefits seem quite obvious.

Younger women, especially those with normal ovarian function, are more likely to have low baseline LH values and are, therefore, at higher risk for profound LH suppression during ovarian down-regulation (Fleming et al., 1998). Since our data demonstrate a beneficial influence of LH-containing stimulation on embryonic diploidy, this benefit should be particularly evident in women who lack sufficient endogenous LH concentrations to (at least partly) compensate for the lack of exogenous LH supplementation during COH. This interpretation of our results is, indeed, supported by a meta-analysis by van Wely et al. (2003a), who, especially in younger women, reported significantly higher clinical pregnancy rates after long agonist stimulation, using hMG, in comparison to FSH.

The likely beneficial effect of hMG stimulation in poor responders, as pointed out by the Cochrane working group (Van Wely et al., 2003b), may also be explained by our findings. The fewer embryos a woman produces, the more detrimental become aneuploidy rates. Whether a woman has a 45% or 70% aneuploidy rate will be especially relevant if she has only few embryos.

The assumed benefit of LH-supplementation in cases of pregnancy loss (Van Wely et al., 2003b) should also not surprise. Diploid embryos are less likely to result in miscarriages (Munne et al., 1995). If LH-containing stimulation results in a higher likelihood of diploid embryos, lower miscarriage rates should follow.

Our results also suggest that the higher pregnancy rates reported with hMG stimulation (Andersen et al., 2006) may be the consequence of improved diploidy rates. Although we observed a higher ongoing pregnancy rate with hMG, the implantation rates were not significantly different between the two groups. If the higher pregnancy rates are further confirmed, the observation would support the basic concept that the availability of more diploid embryos should, at least in properly selected cases, lead to better pregnancy chances (Verlinsky and Kuliev, 1996). This concept has recently been under attack because routine preimplantation genetic screening (PGS) has not been found to be an effective tool in raising pregnancy rates with IVF (Mastenbroek et al., 2007). Our finding is especially remarkable, since they were obtained in PGS cycles, where embryo manipulation does not differ from PGS cycles. They, therefore, suggest that the potential benefit of improved diploidy rates with hMG stimulation fully compensates for the reported decrease in implantation rate that comes from embryo biopsy and embryo mass reduction (Cohen et al., 2007; Mastenbroek et al., 2007), and, actually more than compensates, by adding further to the chance of pregnancy.

In conclusion, the here presented data provide evidence that, at least in selected patient populations, LH-containing ovarian stimulation protocols may beneficially affect diploidy rates in preimplantation embryos.

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References


Cohen J, Wells D, Munne S. Removal of 2 cells from cleavage stage embryos is likely to reduce the efficacy of chromosomal tests that are used to enhance implantation rates. Fertil Steril 2007;87:496–503.


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