Improvement of IVF Outcome in Poor Responders by Discontinuation of GnRH Analogue During the Gonadotropin Stimulation Phase—A Function of Improved Embryo Quality

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Purpose: *To assess the efficacy of a protocol involving the discontinuation of the GnRH analogue at the mid-phase of ovarian stimulation for IVF in patients with a previous poor response.*

Methods: *Prospective case-control evaluation compared with same patient's previous performance. Thirty-six patients enrolled in an IVF program were treated in two consecutive cycles. The first with a standardized protocol utilizing mid-luteal administration of Nafarelin (N) 600 mcg/d continued throughout the stimulation phase with human menopausal gonadotropin (hMG) until follicles of 20 mm were identified by transvaginal ultrasound (Standard group). Patients with a poor response in the Standard cycle were treated in the subsequent cycle with N and hMG initially in a similar manner, then N was stopped after 5 days of hMG stimulation (N-stop group). All clinical and laboratory aspects of treatment were done in a similar fashion in both cycles, each patient acting as her own control.*

Results: *Results were analyzed by paired* t *test. The change in each parameter in the N-stop cycle was expressed as the percent change as compared with the standard protocol cycle for each patient. Peak estradiol (E2) and number of aspirated oocytes were increased in the N-stop cycle (+16.9% and +28%, respectively), but insignificantly so. The percent of cleaving embryos was significantly increased by 27.9% (*p = *0.03) in the N-stop cycle, as embryo morphology was improved by 22% (*p = *0.02). The efficacy of gonadotropin treatment was enhanced in the N-stop cycle, as expressed by a 32.5% increase in oocytes retrieved per hMG ampoule administered (*p = *0.04). Three cycles of 36 were cancelled during the N-stop cycle, whereas only one was cancelled in the standard protocol cycle. Of the 36 patients, 7 conceived in the N-stop protocol and 5 are ongoing pregnancies.*

Conclusion: *Discontinuation of GnRH-a during ovarian stimulation for IVF has a beneficial, but not statistically significant, effect on both E2 and oocyte production. Embryo cleavage rates and morphology were significantly improved, this may be due to improved oocyte quality, which may have been responsible for achieving pregnancies. The efficacy of gonadotropin treatment was enhanced when GnRH-a was discontinued. These results hint that GnRH-a may have a direct negative effect on folliculogenesis and oocytes, which is apparent especially in poor responder patients.*

KEY WORDS: Controlled ovarian hyperstimulation (COH); embryo morphology; gonadotropin-releasing-hormone analogue (GnRH-a); in vitro fertilization (IVF); poor responders.

INTRODUCTION

The introduction of gonadotropin-releasing hormone analogues (GnRH-a) into assisted reproduction technique (ART) protocols some 12 years ago greatly contributed to the success of modern IVF treatment. These medications induce pituitary desensitization, thereby suppressing premature endogenous LH surges, reducing cancellation rates, improving the overall number of oocytes retrieved, and improving implantation rates (1,2). However, other reports indicate that GnRH-a may be responsible for some direct adverse effects on ovarian function, especially steroidogenesis (3,4). Oocyte quality was also

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found to be dependent on GnRH-a concentration in vitro so that high levels of GnRH-a inhibited fertilization and lower levels actually enhanced fertilization (5,6). It might be concluded, therefore, that ovarian responsiveness to gonadotropin stimulation could actually improve if the GnRH-a effect was rescinded during stimulation.

The recovery of pituitary sensitivity after discontinuation of GnRH-a is variable, depending on patients age, dose and mode of administration, and the degree of ovarian stimulation, especially estradiol levels. Although some patients were able to release LH after stopping GnRH-a in as little as 3 days, and primate data demonstrate that the refractory phase does not last more than 6 days (7), the average pituitary recovery time after cessation of nasally administered GnRH-a was found to be 7–10 days (8,9). Discontinuing GnRH-a in mid-stimulation phase would allow this additional time for enhanced stimulation while the pituitary was still refractory. The aim of this study was to examine whether discontinuation of the GnRH-a during controlled ovarian hyperstimulation for IVF would result in an augmented ovarian response, without significantly increasing the cancellation rate, in the same patients.

MATERIAL AND METHODS

Thirty-six patients enrolled in our in vitro fertilization (IVF) program were included in this study. The average age of the patients was 34.7 ± 6.9 years in the range of 30–42 years. The indication for IVF included male factor infertility ($n = 20$), tubal factor infertility ($n = 13$) and unexplained infertility ($n = 3$). The median number of previous IVF cycles was 3, with a 95% confidence interval (CI) of 1–5 cycles. When more than one cycle of treatment was done, the last cycle was chosen for evaluation. Cycle day 3 FSH levels were all below 15 IU/L (range: 4.5–13.2, mean: 8.9 ± 4.2 IU/L). The FSH levels were accepted only if simultaneous serum estradiol levels were <200 pmol/L. These patients were selected after undergoing an initial cycle with the standard Nafarelin–hMG protocol. This entails nasal administration of Nafarelin acetate (Synarel, Delpharm, France) 200 μg per inhalation thrice daily from the mid-luteal phase of a natural cycle, through menses and the stimulation phase until the administration of human Chorionic Gonadotropin (hCG, Chorigon, Teva, Petach Tikva, Israel) 36–40 h before oocyte pick-up. Human Menopausal Gonadotropin (hMG, Pergonal, Teva, Petach Tikva, Israel) was administered starting on the fifth day of menses, with a starting dose of 3 ampoules per day (225 IU) for 4 days and continuing with individually adjusted doses until follicles of 20 mm in diameter were identified by transvaginal ultrasound, when 10000 IU (intramuscular) hCG would be administered. Oocyte pick-up was achieved by transvaginal puncture under general anesthesia. Oocytes were denuded and only mature oocytes (Metaphase II) were injected with spermatozoa, using the intra cytoplasmic sperm injection (ICSI) technique. ICSI was performed in all cycles, in an effort to maximize the number of available embryos for transfer, even when semen analysis did not warrant micromanipulation. Fertilizations were identified after 24 h and cleavage of embryos was assessed after 48 h. Embryo morphology was graded using a standard system including size and uniformity of blastomeres and the degree of fragmentation, as outlined by Veeck (10) and Plachot *et al.* (11), whereby Grade I represents the best morphological status. Grade I: blastomeres of equal size, no cytoplasmic fragments; Grade II: blastomeres of equal size, with minor cytoplasmic fragments or blebs (10–20% of perivitelline space occupied); Grade III: blastomeres of distinctly unequal size, moderate degree of fragmentation (20–50% of perivitelline space occupied); Grade IV: unequal sized blasomeres, more than 50% of embryo surface fragmented, degenerative appearance. Grade I embryos were awarded a score of 1, the next best were awarded a score of 1.5, and so on until Grade IV embryos (the poorest) were given a score of 4.

These 36 patients were selected after reponding poorly in the initial standard cycle. The poor response was defined as either obtaining 5 oocytes or less, or obtaining embryos of poor quality—mean (arithmetical mean of the embryos replaced) score 2.5 or worse—judged by at least two experienced observers (DS and OB or RR or both). The subsequent cycle was similar in every respect to the original standard cycle including the dose and timing of the Nafarelin administration and dose and timing of the hMG. In the "N-stop" cycle, Nafarelin was discontinued after 5 days of hMG administration, and hMG was continued until follicles and estradiol warranted hCG administration.

Parameters measured included days of stimulation, dose of hMG, peak estradiol (day of hCG), the number of oocytes obtained, rate of cleavage of embryos, morphology scores of replaced embryos, the number of cycles cancelled due to premature LH rise, and pregnancy rates. The ovarian response was also assessed by expressing the number of oocytes obtained

and the peak estradiol level as a function of the amount of hMG administered. Scoring of embryo morphology in the subsequent cycle was done using the same criteria and scoring system, and the observer was blinded to the medical protocol used in the same cycle.

STATISTICS

The results of the study were analyzed by paired *t* test: pairs of data in each parameter category were compared between the standard cycle and the N-stop cycle for each patient. The mean change in each category was calculated by expressing the change (delta value) in that parameter from the standard cycle to the N-stop cycle as a fraction of the value in the standard cycle, using the following equation:

$$\Delta(\text{Delta value}) = \frac{\text{value in N-stop cycle} - \text{value in standard cycle}}{\text{value in standard cycle}}.$$

The sum of Δ values for each category (mean delta value) expressed the overall change in that parameter between the two protocols.

RESULTS

In the standard protocol, 1 cycle was cancelled of 36, whereas 3 cycles of 36 (8.3%) were cancelled in the N-stop protocol, due to a premature LH rise before hCG was administered. The average length of the stimulation phase was slightly longer in the standard cycle as opposed to the N-stop cycle—12.6 ± 3.3 days vs. 12.1 ± 5.6 days—but not significantly so. This means that the average length of stimulation without GnRH-a in the N-stop protocol was 7.1 days, thus 33/36 (91.6%) of our patients did not have a premature LH surge before hCG for an average of 1 week without Nafarelin. Luteinizing hormone levels on the day of hCG administration were all less than 6 IU/L except for the three cancelled cycles. There was no difference between the duration of stimulation in the cancelled and noncancelled patients.

When analyzed by group analysis, there were no significant differences between the two protocols in terms of average number of ampoules of hMG per cycle used (45 ± 12 vs. 43 ± 12), peak serum estradiol (1332 ± 800 vs. 1189 ± 770 pg/ml), or number of oocytes aspirated per cycle (5.9 ± 4 vs. 7.2 ± 5.3). Similarly, a trend towards better cleavage rates and embryo morphology was recorded in the N-stop cycle as opposed to the standard cycle when the group was taken as a whole (50 ± 28% vs. 60 ± 31% and 2.19 ± 0.6 vs. 1.8 ± 0.53, respectively), but this trend was not statistically significant. The number of oocytes (in both cycles) obtained was loosely correlated (inversely) with basal FSH levels, but not significantly so (Pearson coefficient of correlation $r = -0.52$).

When data were analyzed by paired analysis (same patient's performance in the standard vs. N-stop cycle), it became apparent that the mean change in response for some parameters, for each patient as an individual was improved (Table I). The number of total ampoules of hMG administered per cycle per patient was only slightly less in the N-stop cycle as opposed to the standard cycle. The level of peak estradiol was increased by a mean of 16.9% per patient in the N-stop cycle as opposed to the standard cycle, but this was not statistically significant. The mean change in the number of oocytes per patient was increased by 28%, (which may be expressed as the equivalent of 1.6 more oocytes per cycle) in the N-stop cycle, this change did not reach statistical significance ($p = 0.08$, paired *t* test). There was a clear positive paired correlation between the number of oocytes obtained in

Table I. Paired Analysis—Comparison of Mean Change (Δ value) of Parameters Between Treatment Cycles

Category[a]	Δ Amps	Δ E2	Δ Oocytes	Δ Cleavg.	Δ Morphl.	Δ O/amp	Δ E2/amp
Mean Δ values	−2.2%	+16.9%	+28%	+27.9%	+22%	+32.5%	+26.8%
(N-stop cycle vs. Standard cycle)[b]	(±0.26)	(±0.88)	(±0.80)	(±0.95)	(±0.37)	(±0.98)	(±0.95)
Significance (*p* value)[c]	ns	ns	ns	$p = 0.03$	$p = 0.02$	$p = 0.04$	ns

Note. Amps: ampoules hMG (75IU FSH & LH/amp) used in cycle; E2: serum estradiol (pg/ml); Cleavg.: cleavage rate of embryos; Morphl.: morpholoy score of embryos. Scale I-IV (see text); O/amp: ratio of number of oocytes obtained per ampoule hMG used in cycle; E2/amp: ratio of estradiol level obtained per ampoule hMG used in cycle. Values in parentheses are standard deviations.

[a] Δ value for each category was calculated by the following formula: Δ value = (value in N-stop cycle – value in Standard cycle)/value in Standard cycle.

[b] Mean Δ value: mean of all patients' Δ values for that category.

[c] Significance was tested by paired *t* test.

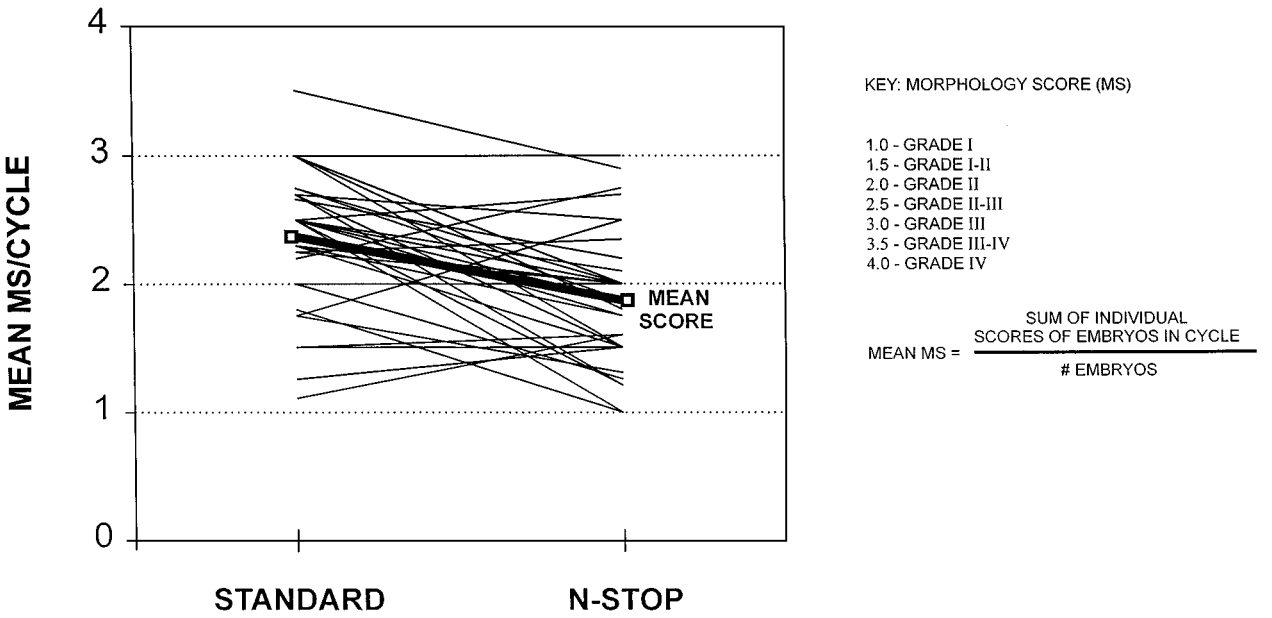

Fig. 1. Embryo morphology.

the Standard as opposed to the N-stop cycle (Pearson correlation coefficient = 0.69). The mean change in embryo cleavage was increased by 27.9% per case in the N-stop cycle, this was statistically significant ($p = 0.03$), and can also be expressed as a mean increase of available embryos of 0.8 embryo per patient. Morphology was also significantly improved—the mean change in morphology score of the replaced embryos for each individual patient was improved by 22% ($p = 0.02$, paired t test). This can also be expressed as an improvement of mean embryo morphology of 0.36 points per patient. (scale of 1.0–4.0). Specifically, mean embryo morphology was improved in 27 patients (75%), unchanged in 3 (8.3%), and worsened in 6 patients (16%; Fig. 1).

The efficacy of gonadotropins was improved by the N-stop protocol, as expressed by an increase in the mean oocyte-obtained per ampoule-hMG-administered ratio of 32.5% ($p = 0.04$, paired t test). A positive paired correlation of 0.7 was noted for this variable, reflecting the obvious improvement in oocyte retrieval despite lower gonadotropin dosage. This can also be expressed as a mean increase of 1.1 oocytes per N-stop cycle if the same amount of gonadotropin was administered, or a mean of seven ampoules *less* administered per cycle to achieve the same amount of oocytes in the same patient in a N-stop cycle. The peak estradiol level in the cycle was analyzed in the same fashion. The mean peak-estradiol per ampoule-hMG-administered ratio in the N-stop cycle as compared with the standard cycle was increased by 26.8% (not statistically significant, $p = 0.057$). This can also be expressed as a mean *decrease* of 3 ampoules per N-stop cycle to achieve the same peak estradiol level.

Clinical pregnancy was achieved in seven of 36 N-stop cycles (35 embryo transfers) as opposed to no pregnancy in the standard cycle, PR = 20% (7/35). Five of these pregnancies resulted in singleton term deliveries with normal neonates, live delivery rate = 14.3% (5/35). All pregnancies were achieved in those patients whose embryo morphology score was improved (5/7) or unchanged (2/7) in the N-stop cycle.

DISCUSSION

GnRH agonist administration is virtually universal in modern IVF stimulation protocols, enabling ovarian stimulation without interference by endogenous pituitary gonadotropin secretion. The addition of these medications has greatly increased ART success rates and as such are associated with extremely low cancellation rates and practically no side effects. Notwithstanding these facts, evidence has accumulated in recent years that GnRH agonists have extrapituitary effects, especially at various sites in the genital tract, in a number of mammalian species (12,13).

GnRH receptors, GnRH receptor mRNA, and receptor binding have been found in endometrial, myometrial, endosalpingeal (14), and placental cells (15). GnRH has been found to have receptors in ovarian granulosa—both follicular phase and luteal cells (16,17)—and theca cells, and in fact have been found in oocytes (18). GnRH production in endometrial cells may have an important role in implantation and in the endometrial-embryonic "cross-talk" (14,19).

The physiology of such receptors is still enigmatic—although, as the systemic levels of GnRH are very low, it is more than likely that GnRH (or GnRH-like peptides) produced locally have a direct or indirect paracrine/autocrine role in regulation of ovarian function (20–23). In vitro studies in mice (5) demonstrated that fertilization rates in the presence of low concentrations of GnRH-a were enhanced, demonstrating a direct effect of GnRH-a on oocytes in an in vitro culture system.

The direct effect of GnRH and its agonists on ovarian steroidogenesis is especially important in the context of ART for poor-responder patients. Some studies failed to show a clear-cut effect of GnRH-a on steroid production in vitro (24,25) where others reported a stimulatory effect at low concentrations of GnRH (3). Gaetje (4) found a dose dependent inhibition of FSH-induced granulosa cell estradiol production by Decapeptyl (D-triptorelin) as opposed to control cultures. Parinaud and colleagues (3) found that GnRH-a (Buserelin) added to luteal phase granulosa cell cultures inhibited LH induced progesterone synthesis. These studies convincingly argue for an inhibitory, negative direct effect of GnRH-a on ovarian folliculogenesis or follicular function, in patients with normal (non-PCOS) ovaries.

Numerous studies have investigated ways to overcome or bypass the direct GnRH-a effect on the ovary. Some authors have tried to reduce GnRH-a doses or to stop its administration during exogenous gonadotropin stimulation to improve ovarian responsiveness, although the results have been contradictory. Higher estradiol levels were achieved with lower doses of gonadotropins, increasing cost-effectiveness, but PR's were unchanged (26,27). Several groups have tried discontinuation protocols with varying success. Hazout (9) utilized a 7-day triptorelin protocol starting on Cycle day 2. When compared with the classic triptorelin 3.75 mg depot form, the discontinuation protocol yielded more embryos per cycle despite markedly decreased hMG requirements, and the cancellation rate was quite low at 2.3%. Pantos and colleagues (28) described their experience with discontinuing subcutaneous Buserelin 0.5 mg/d after 10 days starting in the mid-luteal phase. There were no differences in estradiol levels, hMG dose, or oocytes recovered, although the PR in the discontinuation group was almost twice that of the standard protocol (35.2% vs. 19.4%). There were no cancellations in the discontinuation group, despite up to 12 days without GnRH-a. Sungurtekin and Jansen (29) similarly showed that LH levels were suppressed for 11 days after discontinuation of leuprolide acetate. Fujii *et al.* (30) also studied two groups of patients in a discontinuous GnRH-a protocol, initiating down-regulation in the mid-luteal phase and discontinuing the analogue at Cycle day 7. Interestingly, these authors found that in the discontinuous protocol, more hMG was needed, but less oocytes were fertilized and the cancellation rate was very high (35%). These patients were selected at random from the general IVF population and were not identified as being "poor responders." Faber *et al.* (31) stopped administration of leuprolide at the onset of menses, in conjunction with high dose gonadotropin therapy. Pregnancy rates were improved in this protocol in a group of 182 low responders. Only 1.2% of cycles were cancelled (1/80) because of premature elevation of LH. Another study (32) examined the positive relationship between delay of hMG initiation after depot form GnRH-a and ovarian response and pregnancy rates, this seems to indicate that initiation of gonadotropin stimulation in the presence of reduced concentrations of GnRH-a improves ovarian response and implanation rates. Recently, Dirnfeld and associates examined the effect of a GnRH-a midluteal "stop" protocol in a randomized study (33). One cycle of 40 in the study group was cancelled because of premature LH elevation. A trend towards more oocytes obtained in the study group was found only in those patients previously designated as normal-FSH poor responders; in the general IVF population, no significant differences were demonstrated between the "stop" and the conventional protocol. Conversely, Pinkas and coworkers demonstrated a greater yield of oocytes and subsequently more available embryos for transfer with their "stop" protocol, with no premature LH surges (34).

Our results demonstrate that discontinuation of daily administered GnRH-a in mid-stimulation will not significantly increase cancellation rates due to premature LH surges. This is probably true in a population of poor responders only, and cannot be necessarily extrapolated to include the general IVF population, as Fujii *et al.* (30) found. Our protocol also differed from others in that it continued the Nafarelin

through menses and initiation of stimulation, until the fifth stimulation day. This ensured that the mean treatment period with gonadotropins without GnRH-a coverage was not more than 7 days, and this might be one factor responsible for the low cancellation rate.

Embryo morphology was significantly improved in the N-stop cycle, with each patient acting as her own control. Good quality embryos are significantly associated with improved ongoing pregnancy rates; in fact, embryo quality was found to have a more profound impact on implantation rates in IVF than did age (36–38). As more embryos were available for replacement (mean 0.8 more embryos per patient—2.6 to 3.4 embryos per transfer) and the embryo morphology was improved (by a mean of 0.36 points per patient) in the N-stop cycle, the net result was replacement of one more embryo per patient with a mean morphology score improved by one point. Improved morphology, if all other factors are comparable, is most likely due to improved oocyte quality, which might be attributable to the change in stimulation protocol. Improved oocyte quality and increased fertilization/cleavage rates may also be attributed to lower in vitro levels of GnRH-a in the follicular fluid in the study cycle as opposed to the standard cycle, in accordance with in vitro findings by Yang *et al.* (5). This finding differs from that found by Smitz *et al.* (39) who found that cessation of GnRH-a resulted in poorer quality of supernumerary embryos than did standard protocols. One possible explanation for these differing results might be elevated pre-hCG LH levels in patients in the Smitz study.

Ovarian response was also augmented in the N-stop cycle, as opposed to the previous standard cycle. This was deduced by noting a significantly improved ratio of oocytes-obtained per ampoule-hMG-administered. After optimally controlling for other variables—especially by comparing the same patient's response with the same drugs in a slightly different protocol—this statistically significant improvement can be interpreted as an increased efficacy of gonadotropins at the ovarian–follicular level. Efficacy of gonadotropins in this context, may be expressed as an improved response in terms of estradiol production and an improved oocyte yield, per unit exogenous gonadotropin expended. Improved oocyte yield should be expressed as both more and better oocytes obtained. Our results appear to support the surmise that discontinuation of the GnRH-a during gonadotropin stimulation rescinds an inhibitory effect of the GnRH-a on the ovary, enabling an improved ovarian response. This improvement includes both a more efficacious gonadotropin effect on the ovary, allowing growth of more follicles, and a follicular environment that supports the development of better quality oocytes. It is possible that the discontinuation of GnRH-a also had a beneficial influence on implantation, as GnRH has been found to play a role in embryo-endometrial communication (40). Although the case-control design of this study is not randomized, comparing the same patient's performance in consecutive cycles that differ only in one variable, has the advantage of optimal controlling for differences that might be expressed in randomized patient groups, thereby confounding results. Larger studies could increase the significance of our findings. We believe that patients with poor response in both quantity and especially quality of oocytes could benefit from discontinuing GnRH-a during stimulation, without significantly raising cancellation rates. Further study is underway in in vitro models in an effort to define the direct effect of GnRH-a on granulosa cells and oocytes, to better discern the different patterns of response in different patient groups.

REFERENCES

1. Hugues JN, Cedrin Dunerin I: Revisiting gonadotropin-releasing hormone agonist protocols and management of poor ovarian responses to gonadotrophins. Hum Reprod Update 1998;4:83–101
2. Filicori M, Cognigni GE, Arnone R: Role of different GnRH agonist regimens in pituitary suppression and the outcome of controlled ovarian hyperstimulation. Hum Reprod 1996;11 (Suppl. 3):123–132
3. Parinaud J, Beaur A, Bourreau E, Vieitez G, Pontonnier G: Effect of a luteinizing releasing hormone agonist (Buserelin) on steroidogenesis of cultured human preovulatory granulosa cells. Fertil Steril 1988;50:597–602
4. Gaetje R: Influence of gonadotropin releasing hormone (GnRH) and a GnRH-agonist on granulosa cell steroidogenesis. Clin Exp Obstet Gynecol 1994;21:164–169
5. Yang BC, Uemura T, Minaguchi H: Effects of a gonadotropin releasing hormone agonist on oocyte maturation, fertilization and embryonal development in mice. J Assist Reprod Genet 1995;12:728–732
6. Yoshimura Y, Nakamura Y, Ando M, Shikawa S, Koyama N, Nanno T: Direct effect of gonadotropin releasing hormone agonists on the rabbit ovarian follicle. Fertil Steril 1992;57:1091–1097
7. Winslow KL, Gordon K, Williams RF, Hodgen GD: Interval required for gonadotropin releasing hormone agonist-induced down regulation of the pituitary in cynomolgus monkeys and the duration of the refractory state. Fertil Steril 1992;58:1209–1214
8. Macnamee MC, Howles CM, Edwards RG, Taylor PJ, Elder KT: Short term luteinizing hormone releasing hormone agonist

treatment: Prospective trial of a novel ovarian stimulation regimaen for in vitro fertilization. Fertil Steril 1989;52:264–269
9. Hazout A, de Ziegler D, Cornel C, Fernandez H, Lelaidier C, Frydman R: Comparison of short 7-day and prolonged treatment with gonadotropin releasing hormone agonist desensitization for controlled ovarian hyperstimulation. Fertil Steril 1993;59:596–600
10. Veeck LL: Atlas of the Human Oocyte and Early Conceptus, Vol. 2. Baltimore, Williams and Wilkins, 1991, pp 427–444
11. Plachot M, Junca AM, Mandelbaum J, Cohen J, Salat-Baroux J, DaLage C: Timing of in vitro fertilization of cumulus free and cumulus enclosed human oocytes. Hum Reprod 1986;1:237–239
12. Hsueh AJ, Jones PB: Extrapituitary actions of gonadotropin-releasing hormone. Endocr Rev 1981;2:437–461
13. Fraser HM, Bramley TA, Miller WR, Sharpe RM. Extrapituitary actions of LHRH analogues in tissues of the human female and investigation of the existence and function of LHRH-like peptides. Prog Clin Biol Res 1986;225:29–54
14. Raga F, Casan EM, Bonilla F, Bonilla-Musoles F, Polan ML: Human oviductal gonadotropin releasing hormone: Possible implications in fertilization, early embryonic development and implantation. Fifteenth Annual Meeting of the ESHRE, Tours, France, 1999, Abstract No. 28
15. Minaretzis D, Jakubowski M, Mortola JF, Pavlou S: Gonadotropin releasing hormone receptor gene expression in human ovary and granulosa-lutein cells. J Clin Endocrinol Metab 1995;80:430–434
16. Latouche J, Crumeyrolle-arias M, Jordan D: GnRH receptors in human granulosa cells—anatomical localization and chracterisation by autoradiographic study. Endocrinology 1989;125:1739–1741
17. Brus L, Lambalk CB, de Koning J, Helder MN, Janssens RM, Schoemaker J: Specific gonadotropin releasing hormone analogue binding predominantly in human luteinized follicular aspirates and not in human pre-ovulatory follicles. Hum Reprod 1997;12:769–773
18. Racowsky C, Prather AL, Johnson MK, Overa SP, Gelety TJ: Prematurely condensed chromosomes and meiotic abnormalities in unfertilized human oocytes after ovarian stimulation with and without gonadotropin releasing hormone agonist. Fertil Steril 1997;67:932–938
19. Raga F, Casa FM, Kruessel JS, Bonilla F, Bonilla-Musoles F, Polan ML: Gonadotropin releasing hormone modulation of vascular endothelial growth factor and its transmembrane receptors FLT-1, KDR and sFLT-1: Role in early implantation. 15th Meeting of the ESHRE, Tours, France, 1999, Abstract No. 191
20. Imai A, Takagi A, Horibe S, Takagi H, Tamaya T: Evidence for tight coupling of gonadotropin releasing hormone receptor to stimulated FAS ligand expression in reproductive tract tumors: Possible mechanism for hormonal control of apoptotic cell death. J Clin Endocrinol Metab 1998;83:427–431
21. Kakar SS: Inhibition of growth and proliferation of EcRG 293 cell line expressing high affinity gonadotropin releasing hormone (GnRH) receptor under the control of an inducible promotor by GnRH agonist (D-Lys-6-GnRH) and antagonist. Cancer Res 1998;58:4558–4560
22. Ho MN, Delgado CH, Owens GA, Steller MA: Insulin-like growth factor II participates in the biphasic effect of a gonadotropin releasing hormone agonist on ovarian cancer cell growth. Fertil Steril 1997;67:870–876
23. Guidice LC, Yuan W: Insulin-like growth factor II (IGF-II) mediates the steroidogenic and growth-promoting actions of follicle-stimulating hormone on human pre-antral follicles cultured in-vitro. 15th Meeting of the ESHRE, Tours, France, 1999, Abstract No. 155
24. Dodson WC, Myers T, Morton PC, Conn PM: Leuprolide acetate: Serum and follicular fluid concentrations and effects on human fertilization, embryo growth and granulosa-lutein cell progesterone accumulation in vitro. Fertil Steril 1988;50:612–617
25. Casper R, Erickson G, YenSCC: Studies on the effect of GnRH and its agonist on human luteal steroidogenesis in vitro. Fertil Steril 1984;42:39–43
26. Simon A, Benshushan A, Shushan A: A comparison between a standard and reduced dose of DTRP-6-luteinizing hormone administered after pituitary suppression for in vitro fertilization. Hum Reprod 1994;9:1813–1817
27. Ben Rafael Z, Feldberg D: The poor-responder patient in an in vitro fertilization embryo transfer program. J Assist Reprod Genet 1993;10:118–120
28. Pantos K, Meimeth-Damianaki T, Vaxevanoglu T, Kapetanakis E: Prospective study of a modified gonadotropin-releasing hormone agonist long protocol in an in vitro fertilization program. Fertil Steril 1994;61:709–713
29. Sungurtekin U, Jansen RP: Profound luteinizing hormone suppression after stopping the gonadotropin-releasing hormone agonist leuprolide acetate. Feril Steril 1995;63:663–665
30. Fujii S, Sagara M, Kudo H, Kagiya A, Sato S, Saito Y: A prospective randomized comparison between long and discontinous-long protocols of gonadotropin-releasing hormone agonist for in vitro fertilization. Fertil Steril 1997;67:1166–1168
31. Faber BM, Mayer J, Cox B, Jones D, Toner JP, Oehninger S, Muasher SJ: Cessation of gonadotropin-releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. Fertil Steril 1998;69:826–830
32. Damario MA, Moomjy M, Tortoriello D, Moy F, Davis OK, Rosenwaks Z: Delay of gonadotropin stimulation in patients receiving gonadotropin-releasing hormone agonist (GnRH-a) therapy permits increased clinical efficiency and may enhance in vitro fertilization (IVF) pregnancy rates. Fertil Steril 1997;68:1004–1010
33. Dirnfeld M, Fruchter O, Yshai D, Lissak A, Ahdut A, Abramovici H: Cessation of gonadotropin-releasing hormone analogue (GnRH-a) upon down regulated versus conventional long GnRH-a protocol in poor responders undergoing in vitro fertilization. Fertil Steril 1999;72:406–411
34. Pinkas H, Orvieto R, Avrech OM, Rufas O, Ferber A, Ben-Rafael Z, Fisch B: Gonadotropin stimulation following GnRH-a priming for poor responders in in vitro fertilization-embryo transfer programs. Gynecol Endocrinol 2000;14:11–14
35. Wheeler CA, Cole BF, Frishman GN, Seifer DB, Lovegreen SB, Hackett RJ: Predicting probabilities of pregnancy and multiple gestation from in vitro fertilization—a new model. Obstet Gynecol 1998;91:696–700
36. Hu Y, Maxson WS, Hoffman DI, Ory SJ, Eager S, Dupre J, Lu C: Maximizing pregnancy rates and limiting higher-order multiple conceptions by determining the optimal number of

embryos to transfer based on quality. Fertil Steril 1998;69:650–657
37. Van Kooij RJ, Looman CW, Habbema JD, Dorland M, te Velde ER: Age dependent decrease in embryo implantation rate after in vitro fertilization. Fertil Steril 1996;66:769–775
38. Rosenboom TJ, Vermeiden JPW: Evaluation of embryo scoring systems and their value in predicting in vitro fertilization outcome. Assist Reprod Rev 1995;5:53–59
39. Smitz J, Van den Abbeel E, Bollen N, Camus M, Devroey P, Tournaye H, Van Stierteghem AC: The effect of gonadotropin releasing hormone (GnRH) agonist in the follicular phase of in vitro fertilization outcome in normo-ovulatory women. Hum Reprod 1992;7:1098–1102
40. Raga F, Casan EM, Kruessel J: The role of gonadotrophin-releasing hormone in murine preimplantation embryonic development. Endocrinology 1999;140:3705–3712