CLINICAL ASSISTED REPRODUCTION

Evaluation and Treatment of Low Responders in Assisted Reproductive Technology: A Challenge to Meet

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Purpose: *To investigate the various methods of evaluation and treatment of patients with a low response to controlled ovarian hyperstimulation in assisted reproductive technologies (ART).*
Methods: *Review and analysis of relevant studies published in the last decade, identified through the literature and Medline searches.*
Results: *While a universally accepted definition for low responders is still lacking, these patients are reported to represent about 10% of the ART population. Several ovarian reserve screening techniques have been proposed; however, currently the best-characterized and most sensitive screening tools available are the basal day 3 serum follicle-stimulating hormone level and the clomiphene citrate challenge test. When abnormal, these tests allow physicians to counsel patients that their prognosis for conception is poor. Although the presence of a normal result does indicate better long-term chances for conception, an age-related decline in fecundity remains and patient age should still be considered when counseling patients with normal screening results. Several stimulation protocols have been applied in the low-response group with varying success. Recent studies show that the use of a minidose gonadotropin-releasing hormone-agonist protocol may result in significantly decreased cycle cancellations as well as increased clinical and ongoing pregnancies, and thus is proposed as a first-line therapy. Studies evaluating supplementary forms of treatment to the ovulation induction regimen show improved outcome when pretreating with oral contraceptives, whereas there seems to be no benefit from cotreatment with growth hormone or glucocorticoids. Blastocyst culture and transfer and assisted hatching in low responders are still under evaluation, whereas natural cycle in vitro fertilization may be used in cases of repeated failures as a last option before resorting to oocyte donation or adoption. Future possible forms of treatment like in vitro maturation of immature human oocytes, cytoplasm, and nuclear transfer currently are experimental in nature and their efficacy has still to be proven.*
Conclusions: *The evaluation and treatment of low responders in ART remains a challenge. Understanding of the underlying etiology and pathophysiology of this disorder may help the clinician to approach it successfully.*

KEY WORDS: Assisted reproductive technologies; low responders; ovarian response, ovulation induction.

The application of gonadotropin administration to stimulate multiple follicular recruitment and the subsequent transfer of multiple embryos has dramatically increased the success of assisted reproductive technologies (ART). However, a number of women are found to respond poorly or not at all to this treatment. Such patients, whose incidence is estimated to be about 10% of the ART population, often are referred to as "low responders" and are unable to take full advantage of ART (1). Controlled ovarian hyperstimulation (COH) in low responders may result in cycle cancellation, insufficient response with poor follicle recruitment in number or size, low levels, slow increase or drop in serum estradiol (E_2), and low pregnancy rates (2). This phenomenon was first described by Garcia *et al.* (3) in 1983, in patients who attained a peak E_2 level of $<$ 300 pg/ml after standard stimulation with human menopausal gonadotropins (hMG).

While a universally accepted definition is not currently available, most agree that patients who have produced less than three to four oocytes or have had

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a low peak E_2 level [upper limit at 500 pg/ml or even 1000 pg/ml on the day of human chorionic gonadotropin (hCG) administration] in a previous stimulation cycle could be characterized as low responders (4). Also, some designate maternal age $\geq$ 40 years as a salient factor in the low response category (5). Low response to COH frequently reflects an age-related decline in reproductive performance, but the same phenomenon may occur in young patients (6). Some women have an occult ovarian failure, evidenced by a basal or clomiphene-induced increase in serum follicle-stimulating hormone (FSH) levels, whereas others have normal serum FSH and no apparent reason for repeated low responses to aggressive stimulation protocols (7). Several mechanisms have been proposed to explain low response in these women with normal age and FSH; however, none of these hypotheses has ever been proved, suggesting that these patients also may have diminished ovarian reserve (8).

Since the evolution of ART, the management of these infertile patients has been one of the most difficult challenges and disappointing issues in reproductive medicine. It is the purpose of this review to summarize the proposed methods of evaluation and currently available treatment strategies of low responders in ART. The possibility of applying micromanipulation techniques in order to overcome oocyte aging will be discussed.

PREDICTION OF OVARIAN STIMULATION OUTCOME

Identifying low responders prior to initiation of hormonal treatment for in vitro fertilization–embryo transfer (IVF-ET) is extremely important, so that the patients can be counseled regarding the lower chances for pregnancy, have realistic expectations, and consider alternative therapies such as oocyte donation or adoption. In addition, stimulation protocols for these patients can be modified to lower the risk of cancellation and improve pregnancy rates. As a result, numerous screening methods have been proposed to assess prospectively ovarian reserve and to individualize treatment regimens.

Age

Ovarian reserve decline has been clearly related to age; however, the prognostic value of age alone has not yet been established (9). An earlier cohort study of artificial insemination by donor (10) and a recent multicenter study of IVF (11) showed that the probability of success of ART decreased progressively after the age of 30 years and declined markedly after the age of 35 years. A similar decreasing trend in the residual follicle count within both ovaries has been demonstrated, as ovarian follicle atresia is accelerated after the age of 38 years (12). In accordance to these, Lass *et al.* (13), who recently evaluated 1087 IVF cycles initiated in 471 women $\geq$ 40 years of age, reported that the pregnancy rate was significantly lower in these women than in a control group of women $<$ 40 years of age (11.3% vs. 28.2%, respectively). The pregnancy rate decreased sharply in women $>$ 42 years of age, and no women $>$ 45 achieved a live birth (13). However, in the latest Human Fertilization and Embryology Authority Report, a 10.4% live birthrate per treatment cycle was reported for 96 women of $\geq$ 45 years of age undergoing ART treatment (14). Also, for women $>$ 39 years of age, a 10.7% and 7.0%, pregnancy and live birthrate per treatment cycle, respectively, were reported in the 1996 ART Report of the American Society for Reproductive Medicine (15). Thus, it seems that age alone is not the only outcome-determining factor for patients undergoing ARTs, nor is it a reliable predictor for patients over or under 40 years of age.

Hormonal Tests of Ovarian Reserve

Several hormone biomarker tests of ovarian reserve have been reported (Table I). The most commonly used is the serum FSH assay on cycle day 3, which has proved to be highly predictive of diminished ovarian reserve, as defined by poor gonadotropin responsiveness and pregnancy rates in patients undergoing ART. Toner *et al.* (16) reported on a series of 1478 consecutive IVF cycles that basal day 3 FSH levels screening showed that the cancellation rates for patients with levels of $<$15 mIU/ml, 20 mIU/ml, 25 mIU/ml, and $>$ 30 mIU/ml were 5%, 10%, 20%, and 40%, respectively. Also, in a retrospective study of 758 IVF cycles, Scott *et al.* (17) found that pregnancy rates decrease markedly as basal day 3 FSH levels increased. Specifically, women with serum FSH $<$ 15mIU/ml had a pregnancy rate double that of women with levels of 15 to 24.9 mIU/ml and sixfold higher than that of women with a concentration of $\geq$25 mIU/ml. This reduction in pregnancy rates was attributed to decreased ovarian reserve because these patients produced fewer follicles, had fewer oocytes aspirated, and had fewer embryos transferred.

Table I. Available Hormone Screening Tests for Assessing Ovarian Reserve

Reference	No. of cycles	Screening test	Values	Cancellation rate per initiated cycle(%)	Pregnancy rate per initiated cycle (%)
Scott et al. (17)	758	Basal day 3 FSH	<15 IU/liter	50/541 (9.2)	130/541 (24.0)
			15 to 24.9 IU/liter	37/161 (22.9)	22/161 (13.6)
			≥25 IU/liter	18/56 (32.1)	6/56 (10.7)
Toner et al. (16)	1478	Basal day 3 FSH	<20 IU/liter	130/1305 (10.0)	196/1305 (15.0)
			≥25 IU/liter	20/80 (25.0)	0/80 (0)
Mukherjee et al. (19)	74	Day 3 FSH:LH Ratio	≤3.6	2/60 (3.3)	15/60 (25)
			<3.6	12/14 (85.7)	0/14 (0)
Smotrich et al. (20)	292	Day 3 serum estradiol	<80 pg/ml	1/265 (0.4)	98/265 (37)
			≥80 pg/ml	5/27 (18.5)	4/27 (14.8)
Licciardi et al. (21)	452	Day 3 serum estradiol	≤75 pg/ml	NA[c]	86/422 (20)
			>75 pg/ml	NA	0/30 (0)
Loumaye et al. (24)	114	CC Challenge Test (FSH)	≤26 IU/liter[a]	1/94 (1)	26/94 (27.6)
			>26 IU/liter[a]	5/20 (25)	0/20 (0)
Tanbo et al. (23)	165	CC Challenge Test (FSH)	<12 IU/liter	35/111 (31.5)	11/111 (9.9)
			≥12 IU/liter	46/54 (85.2)	0/54 (0)
Winslow et al (26)	228	GnRH-a Stimulation Test	$\Delta E2^b > 15$ pg/ml	NA	52/211 (24.6)
			$\Delta E2^b \leq 15$ pg/ml	NA	1/17 (6)

[a] Represents the sum of day 3 and 10 FSH levels.
[b] Change in estradiol on day 3 over the baseline day 2 value.
[c] NA, not available.

In an effort to further characterize the ovarian reserve, other markers of hypothalamic–pituitary–ovarian axis function were examined. In the natural menopause, FSH and luteinizing hormone (LH) levels increase with diminishing ovarian reserve. Lenton *et al.* (18) demonstrated that serum FSH increases several years before elevations in serum LH, and as a result the first intimation of a diminished ovarian reserve may be an elevated FSH:LH ratio. Mukherjee *et al.* (19) applied this finding in women of reproductive age undergoing COH for IVF-ET and have shown that in patients with a normal day 3 FSH an FSH:LH ratio > 3.6 had a sensitivity of 85% and a specificity of 95% for predicting a poor response to COH.

A day 3 serum E_2 determination also has been proposed as a means of assessing reproductive potential in women prone to undergo IVF. Studies have shown that increasing levels of day 3 E_2 are related with a decrease in the number of oocytes retrieved and pregnancy rates. Specifically Smotrich *et al.* (20) demonstrated that day 3 E_2 levels > 80 pg/ml in a cycle proximate but before performing IVF strongly and independently predicted poor ovarian response and clinical outcome, as these patients had a higher cancellation rate and achieved a lower pregnancy rate. Patients with day 3 E_2 concentration ≥ 100 pg/ml achieved no pregnancies. It should be noted that their data also confirmed the utility of the day 3 FSH levels as a predictor. Similar results were reported by Licciardi *et al.* (21), who proposed that the combination of day 3 FSH and E_2 values might improve the prognostic ability of either of these hormones used alone.

The variation of basal FSH in different cycles and the fact that some poor responders have a normal day 3 FSH led to the proposal of ovarian performance predictive tests. Navot *et al.* (22) proposed the clomiphene citrate (CC) challenge test as a means of assessing ovarian reserve in women ≥ 35 years of age. Simply, serum FSH determinations are obtained on cycle day 3 (basal) and then again on cycle day 10 (stimulated) after the administration of 100 mg of CC during days 5 to 9. An abnormal test is defined by an elevated value in the day 10 (FSH > 10 IU/liter) sample compared with that of day 3. Obviously, an abnormal value on cycle day 3 also results in the test being considered abnormal.

Several groups evaluated the predictive value of CC challenge test screening in patients participating in ART programs. Tanbo *et al.* (23) studied 91 women over age 35 undergoing IVF and found abnormal CC challenge tests in 37. The predictive value of an abnormal test was 85% for cycle cancellation due to poor ovarian responsiveness and 100% for failing to conceive. In contrast, cancellation rates were much lower (31.5%) and pregnancy rates much higher (11%) in those patients with normal tests. Several other studies reported similar results, confirming the excellent predictive value of the CC challenge test for diminished ovarian reserve and poor long-term pregnancy rates in

natural cycles, during ovulation induction, and in IVF (24, 25).

The Norfolk group proposed the gonadotropin-releasing hormone-agonist (GnRH-a) stimulation test, which evaluates the change in E_2 level from cycle day 2 to 3 after the administration of 1 mg of leuprolide acetate (26). The group described a strong correlation between the change in E_2 level and several parameters of IVF success and advocated the test for selected patients undergoing IVF. In particular, patients showing at least a doubling of their baseline E_2 level on day 3 compared with that on day 2 achieved a higher number of oocytes retrieved and pregnancy rates. On the other hand, in patients who experienced less than a doubling of their E_2 level on day 3 compared with that on day 2, the cycle cancellation rate was higher, whereas the number of oocytes retrieved and the pregnancy rates were lower.

Scott *et al.* (27) in a recent review of the literature proposed that currently the best characterized and most sensitive screening tool available is the CC challenge test. This test may be superior to basal FSH screening; however, further data are needed in order to recommend omission of the day 3 FSH sample, and thus currently the majority of ART programs still continue to screen their patients with both day 3 and day 10 FSH levels (27).

Ultrasonographic Evaluation

Despite the implementation of the above-described hormone tests, the 1993 Annual Report for the Society for Assisted Reproductive Technologies (28) documented a cancellation rate of 14% per initiated IVF cycle. Thus, other means of evaluating ovarian response were investigated.

Recent studies have found a good correlation between ovarian response to stimulation and ultrasonographic findings, providing the rationale for wide use of this noninvasive technique. Zaidi *et al.* (29) investigated the relationship between ovarian stromal blood flow to subsequent follicular response and found that mean ovarian stromal peak systolic blood flow velocity was significantly lower in the low response group and that the odds of a poor response decreased significantly as peak systolic blood flow velocity increased. They proposed that the measurement of ovarian stromal blood flow before ovarian stimulation might be a new indicator for predicting subsequent ovarian responsiveness (29). Oyesanya *et al.* (30) reported that follicular vascularity index (ratio of the number of follicles with demonstrable pulsatile vascularity to the total) correlated positively with oocyte recovery rates, and thus proposed that detection and quantification of follicular vascularity with color Doppler imaging may be used to predict oocyte recovery rate. Other studies also have reported higher pulsatility and resistance indices in low responders compared to normal controls (31, 32). Chang *et al.* (33) recently reported a clear correlation between the number of antral (2–5 mm) follicles under basal conditions and the outcome of ART. A total of 149 treatment cycles for 130 couples were performed, and the number of antral follicles with a diameter of 2–5 mm was determined on the first or second day of menstruation or just before the administration of gonadotropins. The number of antral follicles correlated significantly with patient age, day 3 serum FSH level, dosage of menotropin ampules used, serum E_2 concentration, number of oocytes retrieved, and later number of oocytes or embryos transferred. The group of patients who had a lower antral follicle count ($\leq$ 3) also had a significantly higher rate of cycle cancellation compared with the other groups who had a higher antral follicle count. The introduction of three-dimensional ultrasonography as a diagnostic instrument in reproductive medicine has added a new dimension to ultrasound examination of the ovary. Using this approach, the reproducibility of ovarian volume measurements has already been proved as well as its efficacy in the diagnosis of ovarian masses (34). Moreover, it is possible to document follicles measuring 2–3 mm, as well as the process of follicular selection and dominance (35). Based on these findings, Pellicer *et al.* (36) recently proposed the introduction of three-dimensional ultrasonography in the evaluation of ovarian reserve in young low responders with normal basal levels of FSH and showed that low responders exhibited a statistically significant decrease in the total number of follicles and in the number of selectable follicles as compared with normal responders.

Data reported at the present time suggest that ultrasonography may be a useful noninvasive tool in the prediction of ovarian reserve and ovarian response to stimulating drugs; however, future standardization of the criteria used is necessary in order to achieve its clinical routine application (37).

OVULATION INDUCTION PROTOCOLS IN LOW RESPONDERS

The ideal approach to patients who respond poorly to traditional controlled ovarian hyperstimulation regimens remains one of the major challenges in ART.

Several stimulation protocols have been described with varying success (Table II).

CC-hMG Cycles

Clomiphene citrate has been widely used in combination with hMG to induce multiple follicular development at the inception of IVF. Its use in ART has been limited following the introduction of GnRH-a/hMG protocols, which were found to lead to a greater number of retrieved oocytes, increased pregnancy rates, better scheduling, and lower cancellation rates (38). Clomiphene citrate/hMG regimens still seem to have an important role in the treatment of low responders. Dor *et al.* (39), in a retrospective study of 1099 IVF cycles treated with GnRH-a/hMG, hMG, or CC/hMG have shown that GnRH-a-containing protocols provided significantly lower cancellation rates. However, in women over 40, GnRH-a/hMG resulted in the highest rate of poor ovarian response. Despite more oocytes retrieved, fertilized, and cleaved after the use of GnRH-a/hMG, the clinical pregnancy rate was the highest with CC/hMG compared with the other two treatments (39).

Benadiva *et al.* (40) recently have studied the effect of CC/hMG in 93 patients who have had previously failed IVF attempts using gonadotropins with or without GnRH-a. Cancellation rate, length of stimulation, and peak E_2 levels, did not differ significantly between the two stimulating regimens, although more ampules of hMG were required in the non-CC cycles to achieve similar results. However, the implantation rate per embryo and live birthrate per transfer were significantly higher in the CC/hMG cycles than in the previous gonadotropin-stimulated cycles (13.6% and 26.2% vs. 0.7% and 0%, respectively). These researchers concluded that low-response patients may benefit from the addition of CC to their ovarian stimulation protocol, and that this beneficial effect may be related to an enhanced embryo quality as suggested by a higher cleavage rate and a greater implantation rate per embryo. In a well-controlled study, utilizing CC/hMG in a poor-responder group of 51 patients previously cancelled on a high-dose hMG protocol, Pellicer *et al.* (1) found that certain subgroups (separated according to the pattern of the E_2 profile) responded better with this protocol compared to hMG protocols for IVF. Similar conclusions were reached by Ben-Rafael *et al.* (41) who proposed that in elderly poor responders CC/hMG is cost-effective and should be the first-line attempt. Also, in a retrospective study of 271 patients, Pantos *et al.* (42) studied the effect of increasing the hMG dose in a CC/hMG hyperstimulation protocol for IVF and found that increasing hMG dosage above 150

Table II. Stimulation Regimens in the Treatment of Low Responders

Stimulation regimen	No. of cycles	Age (years)	Days of stimulation	Ampules of gonadotropins	Cancellation rate (%)	Mean no. of oocytes retrieved	Clinical pregnancies per retrieval (%)	Ovarian response	Pregnancy rate
CC/hMG									
Pellicer et al. (1)	95	NA[a]	NA	NA	82.0	2.8 ± 1.4	NA	Not improved	NA
Pantos et al. (42)	271	NA	NA	NA	10.7	6.0[b]	NA	Not improved	NA
Benadiva et al. (40)	93	36.2 ± 3.3	10.6 ± 1.5	12.1 ± 5.6	24.7	5.8 ± 4.0	13.6[c]	Not improved	Improved
High-dose hMG									
Hofmann et al. (47)	23	36.1 ± 3.3	NA	28.0 ± 6.0	9.0	2.6 ± 0.9	34.0	Not improved	Improved
Stadtmauer et al. (50)[d]	350	36.4 ± 0.4	10.5 ± 0.3	49.1 ± 1.4	24.6	9.0 + 0.5	34.0	Improved	Not improved
Land et al. (51)	126	34.0	11.2 ± 1.6	67.1 ± 6.7	28.6	7.5 ± 4.5	4.4	Improved	Not improved
GnRH-agonists									
Long protocol									
Jenkins et al. (57)	80	35.3 ± 4.4	11.0	NA	23.7	8.9	9.0	Not improved	Not improved
Short protocol									
Padilla et al. (60)	53	35.0 ± 3.5	NA	NA	11.3	7.3 ± 4.5	31.9	Not improved	Improved
Karande et al. (63)	80	37.2 ± 3.9	NA	NA	23.8	10.0 ± 6.6	11.5	Improved	Not improved
Minidose GnRH-a protocol									
Scott and Navot (66)	34	37.9 ± 0.4	9.6 ± 0.2	26.8 ± 0.3	0.0	5.1 ± 0.2	8.8	Improved	NA
Feldberg et al. (67)	36	37.9 + 1.9	11.7 ± 1.1	36.9 ± 6.1	11.1	4.1 ± 1.3	28.1[c]	Improved	Improved
Schoolcraft et al. (69)	32	36.8 ± 3.6	NA	53.0 ± 10.5	12.5	10.9 ± 4.2	50.0[c]	Improved	Improved
Surrey et al. (68)	34	38.9 ± 0.4	11.9 ± 0.7	74.7 ± 6.4	20.3	5.3 ± 0.7	34.8[c]	Not improved	Improved

[a] NA, Not available.
[b] Median number of eggs retrieved.
[c] Number of pregnancies/number of embryos transferred.
[d] Study included normal and low responders.

IU does not appear to increase the number of oocytes retrieved. They concluded that a poor response might be due to inherent differences in follicular development that cannot be overcome by increases in hMG dosage.

The effect of low-dose CC stimulation alone in IVF in women who respond poorly to superovulation also was recently studied by Awonuga *et al.* (43). The clinical pregnancy rates per oocyte collection achieved in the first CC cycle in nonresponders (9.1%) and poor responders (10%) were comparable to those achieved by poor responders (11.9%) who had GnRH-a/hMG using the long protocol. They concluded that for such poor responders, three attempts of IVF in a CC cycle might offer a viable therapeutic alternative before reverting to more stressful, expensive, and time-consuming treatment.

It may be concluded that despite the higher risk of cancellation rate and the possible nondesirable effects of CC on the reproductive tract, CC/hMG regimens seem to be cost-effective and should be considered in the treatment of low responders.

High-Dose Gonadotropins (hMG or FSH or hMG/FSH) Protocol

One of the initial approaches in the treatment of low responders was to increase the dose of gonadotropin administered, starting on cycle day 1–3, with the expectation that these patients would respond to the higher serum levels of gonadotropins with an increase in the number of oocytes recruited. Unfortunately, endogenous LH levels were suppressed and premature luteinization and ovulation continued to be a risk with this stimulation and required frequent monitoring of LH (44).

In an early study, Laufer *et al.* (45) used a relatively high dose of hMG in their stimulation regimen in an effort to enhance pregnancy rates. Although at least two cleaved embryos were obtained in more than 80% of the cycles, their pregnancy rate was comparable to that described previously with a more conservative approach (46), raising doubts about the cost-effectiveness of the high-dose hMG treatment. In another study, Hofmann *et al.* (47), who evaluated the effect of high-dose FSH (6 ampules/day) ovarian stimulation in low-responder patients for IVF, reported that while there was no change in the number of oocytes retrieved, fertilized, or transferred, the cancellation rate was reduced, whereas the pregnancy rate was improved.

However, other groups have shown lower pregnancy rates in patients receiving high dosages of gonadotropins. In a group of IVF patients receiving CC and hMG, no pregnancies resulted in cycles where the starting dose of hMG was $>$ 300 IU/liter (48). Karande *et al.* (49), who used six ampules of FSH daily in 34 poor responder patients, also showed that increasing the dosage of medication does not lead to increased pregnancy rates and observed no significant difference with respect to peak E_2, number of preovulatory oocytes retrieved, or number of embryos replaced. Similarly, Stadmauer *et al.* (50) reported lower pregnancy rates in IVF-ET with high dosages of exogenous gonadotropins, suggesting a possible deleterious effect of excess hMG on the ability to establish a pregnancy.

Recently, Land *et al.* (51), in an attempt to improve the outcome in poor responders, doubled the dose of hMG in the second cycle. While a significant increase in the number of eggs retrieved was achieved, the number of embryos and the pregnancy rate did not differ significantly. Similarly, Rombauts *et al.* (52), who studied the effectiveness of recombinant human FSH therapy in 40 low-response patients, found no additional benefit from its use.

It may be concluded that at higher doses, some improvement in response may be achieved, but at some point saturation kinetics are attained, which place a limitation on the effectiveness of this form of therapy.

GnRH-Agonists Protocols in Low Responders

A variety of GnRH-a protocols have been developed since the 1980s, and their use has gained widespread popularity for ovarian stimulation since then. Some prospective studies showed no difference in pregnancy rates when either a long or a short protocol is used (53, 54). However, several retrospective studies and large prospective studies demonstrated that the long GnRH-a protocol is superior to the short protocol in terms of follicular recruitment, oocyte recovery, fertilization rates, and number of embryos (55, 56). This effect has been attributed to the abolition of premature LH surges and to synchronization of follicular recruitment as a result of pituitary desensitization.

Unfortunately, prior suppression, according to the long protocol with GnRH-a, in low responders has been found to result in excessive dampening of the ovarian response to hormonal stimulation, so that cancellation rates, due to lack of ovarian response, are unacceptably high or hormonal stimulation is excessively prolonged with increased cost and duration of treatment without a significant improvement in the yield of mature oocytes (5). Jenkins *et al.* (57) reported that 19 of 80 (24%) poor responders down-regulated

with Buserelin acetate and stimulated with 300 IU of hMG daily did not reach oocyte retrieval.

The short or "flare-up" GnRH-a protocol has been proposed as a better stimulation protocol for low responders as this form of treatment takes advantage of the initial stimulatory effect of GnRH-a action on pituitary hormone levels, and thus can be employed to improve the stimulatory response of low responders (58, 59). Muasher (5) reported a low cancellation rate (5%) in 150 cycles of poor responders using a flare-up GnRH-a protocol starting on cycle day 2 and four to six ampules of FSH starting on day 3. Padilla *et al.* (60), who evaluated the flare-up protocol with high-dose FSH and hMG for IVF in poor responders in comparison to good responders, reported a significantly higher cancellation rate in the former group than the latter (11.3% and 1.1%, respectively). In addition, lower peak E_2 levels, decreased number of oocytes retrieved, and number of embryos available for transfer characterized the low-response group. Importantly, however, the fertilization and pregnancy rates for the patients who had responded were the same as in good responders (60). Also, Yang *et al.* (61) showed that in patients with FSH:LH ratios, either ≥ 3.0, ≥ 2.5, or ≥ 2.0, the pregnancy rates were higher when using the short than the long protocol. An important drawback in using the short protocol is that patients who receive GnRH-a in this early phase will have an increase in gonadotropins that also may induce enhanced ovarian androgen release, corpus luteum rescue, and a secondary decline in oocyte quality and ongoing pregnancy rates compared with those who receive GnRH-a in the midluteal phase (62). Karande *et al.* (63), in a prospective study of 80 poor-response patients treated with the flare-up protocol, reported that while its application often will lead to an adequate ovarian response, oocyte retrieval, and embryo transfer, the pregnancy rate remains low (6.5% per retrieval). Nevertheless, experience to date shows that the short protocol has an important role in the treatment of low responders and may be used as first-line therapy.

Other protocols for low responders also have been developed. The ultrashort protocol, as the short protocol, takes advantage of the initial surge of gonadotropin secretion induced by the GnRH-a, which is administered for only 3 days in the early follicular phase, days 2, 3, and 4. This protocol has proved to be advantageous compared with the long protocol in respect to the length of the treatment cycle and the amount of menotropin needed. However, premature ovulation and cycle cancellation are still troublesome with the ultrashort protocol compared with short and long protocols. We use the ultrashort protocol as a last resort for low responders who do not produce adequate follicular growth with other modes of stimulation (64).

Recently, Faber *et al.*, (65) proposed a new protocol for low responders in whom the GnRH-a was initiated in the midluteal phase, terminated by the onset of menses, and then followed by high-dose gonadotropin therapy. A 12.5% cancellation rate was reported as a result of inadequate response. However, the majority of the patients produced an adequate number of mature oocytes (approximately 10 oocytes per stimulation attempt), achieved three or more embryos per transfer resulting in an acceptable clinical pregnancy rate of 32% per transfer (65).

Minidose GnRH-a Protocol

In 1994, Scott and Navot (66) offered a novel approach to the poor responder with the introduction of a microdose GnRH-a flare protocol, after oral contraceptive pretreatment. They found that approximately 2% of the normal dose of GnRH-a (20 μg leuprolide acetate, BID) was able to stimulate significant endogenous gonadotropin release and to inhibit premature LH surges in 100% of cases. Compared with a luteal GnRH-a protocol, this microdose GnRH-a flare protocol improved stimulation outcome in 90% of cases.

In accordance to these results, Feldberg *et al.* (67) studied minidose luteal phase GnRH-a [Decapeptyl ($D\text{-}Trp^6$, Ferring, Malmo, Sweden)] with hMG administration in poor responders with elevated basal FSH levels. They found that patients who received 0.1 mg/day of agonist from the midluteal phase until menstruation and then 0.05 mg/day thereafter had higher peak E_2 levels, increased number of oocytes recovered, and higher number of embryos transferred compared with patients who were treated with higher Decapeptyl daily doses. They also noted a trend toward improved pregnancy rates and implantation rates and a lower spontaneous abortion rate. Surrey *et al.* (68) recently studied the clinical and endocrine effects of a minidose GnRH-a flare regimen administered to poor responders who are undergoing IVF, and found significantly improved results by the application of this regimen compared to a standard GnRH-a long protocol. Cycle cancellation rates were significantly decreased, and the percentage of patients able to undergo embryo transfer was significantly increased with this approach regardless of patient age. Peak serum E_2 levels were greater with the use of the microdose regimen, but there was no evidence of premature luteinization as manifested by

uniformly low serum progesterone levels on the day of hCG administration.

In a study of 32 poor-responder patients who were pretreated for 21 days with oral contraceptives followed by the administration of a 40-μg dose of leuprolide acetate twice daily simultaneously with growth hormone (4 IU/day intramuscularly), Schoolcraft *et al.* (69) reported a 12.5% cancellation rate and an impressive 50% ongoing pregnancy rate per oocyte retrieval in a group of patients with a previously poor response.

In summary, these studies show that the use of a microdose GnRH-a protocol as part of a COH regimen for poor responders who are candidates for IVF-ET may result in significantly decreased cycle cancellations as well as increased clinical and ongoing pregnancy rates. The mechanism by which this improved outcome is achieved has not been clearly verified, but it may involve an enhanced release of early follicular phase FSH without concomitant increase in circulating LH or progesterone levels.

SUPPLEMENTARY FORMS OF TREATMENT TO THE OVULATION INDUCTION REGIMENS

Growth Hormone

Growth hormone (GH) has been recognized as having a possible role in the regulation of ovarian follicular development and has been clinically investigated for its use in intensifying the ovarian response to hMG for ovulation induction. Initial studies have shown that GH may enhance the gonadotropin effects on granulosa cells in culture, either via locally acting insulinlike growth factor-I (IGF-I) or by direct GH action, and thus, its inclusion in stimulation regimens might lead to a shorter duration of treatment, a lower total dose of required gonadotropins, and a possible increased response (70).

Homburg *et al.* (71) have suggested that cotreatment with biosynthetic human GH sensitizes the human ovary to the stimulatory effect of treatment with gonadotropins. They followed their study by a randomized, double-blind, placebo-controlled trial in a group of patients with hypogonadotropic hypogonadism that reconfirmed the positive role of GH in the augmentation of ovarian response. Thus, it was assumed that a major application of GH supplementation might be in improving the outcome of treatment in women with poor ovarian response.

Several studies, therefore, have focused on this issue. Volpe *et al.* (72) found an amplification of the gonadotropic effect following GH treatment in patients resistant to ovulation induction. This effect was demonstrated only in young patients aged 25–34 years, while older patients aged 35–41 years did not show any significant improvement of their ovarian response following combined GH/gonadotropin treatment and all their stimulatory cycles were cancelled. Bergh *et al.* (73) reported that, although GH did not increase oocyte numbers or peak E_2, it did significantly increase the fertilization rate per oocyte and therefore the yield of embryos for subsequent transfer, a finding that may be clinically important in a group of patients like the low responders from which very few oocytes are retrieved. However, further studies failed to demonstrate an improved clinical response following the addition of adjuvant GH therapy to ovulation induction with the use of exogenous gonadotropins. In a large, randomized, well-controlled study, Hughes *et al.* (74) failed to elicit any clinical advantage of the addition of GH to poor responders receiving GnRH-a/hMG undergoing IVF therapy. In a double-blind placebo-controlled study using a flare-up protocol for ovarian stimulation, Suikkari *et al.* (75) demonstrated no improvement in cycle outcome with daily adjuvant human GH administration provided in doses of 4 or 12 IU.

There is a difficulty in comparing the results of these studies because of heterogeneity in patient selection, dosages of GH and regimens used, and the definitions of low ovarian response. However, from the data reported thus far it may be concluded that poor IVF responders do not seem to benefit from cotreatment with human GH during their ovarian stimulation.

Growth hormone-releasing hormone (GHRH) also has been studied as an adjuvant tool in ovulation induction in low-responder patients. While results reported thus far are conflicting regarding its efficacy, Busacca *et al.* (76), in a recent prospective randomized trial, found that GHRH used with FSH to induce follicular development in low responders resulted in an increased number and size of follicles, but no difference was noted in pregnancy rates between the GRF-treated and control patients.

Glucocorticoids

Glucocorticoids have been shown to have a beneficial effect in selected patients during COH. Studies of patients enrolled in IVF-ET programs suggested an indirect effect of cortisol on the ovaries and an essential

role of cortisol in the maintenance of granulosa cell cultures. The proposed mechanisms of action include a direct action of glucocorticoids on the ovaries, on the gonadotropin production at the pituitary level, and by suppressing the adrenal androgens. Adjuvant glucocorticoid therapy to the treatment of low responders also has been investigated, however, with disappointing results. There seems to be no enhancement of the ovarian response or improvement in the pregnancy rate. However, individual patients, such as those in a hyperandrogenemic state and patients in stress, in specific circumstances may benefit from glucocorticoid supplementation due to its effect on the ovaries, adrenals, and pituitary glands (77).

Oral Contraceptives

Suppression of the pituitary–ovarian axis may also be achieved by the use of oral contraceptives (OC). Induction of a transient hypoestrogenic state by OC before commencing COH treatment has been recommended for improving the prediction of the timing of follicular aspiration. Moreover, Gonen *et al.* (78) have demonstrated that pretreatment of an IVF cycle with OC may be associated with a reduction in the amount of gonadotropins required for ovarian stimulation and with a higher yield of aspirated oocytes.

Scott and Navot (66) pretreated poor responders with OC before applying their minidose GnRH-a flare-up protocol and reported significantly improved outcome. Using a similar approach, Schoolcraft *et al.* (69) reported improved COH in poor responder IVF patients by applying a precycle treatment with OC followed by a microdose FSH flare-up growth hormone protocol. They proposed that pretreatment of each cycle with OC possibly was a significant factor in the successful clinical outcome seen, and this could be explained by the fact that by eliminating the existence of a corpus luteum from the prior cycle, the flare effect of GnRH-a was unable to rescue a preexisting corpus luteum and its associated premature progesterone production. Fisch *et al.* (79) pretreated 42 low responders with OC for 28–42 days followed immediately by gonadotropin stimulation and compared the results with those obtained in previous stimulation cycles of the same women with the CC/hMG regimen. Significantly higher peak E_2 levels and number of preovulatory follicles were noted in the former group. The cancellation rate (34% vs. 75%), the number of oocytes retrieved (6.1 ± 3.0 vs. 2.4 ± 1.3), and the pregnancy rate (16.1% vs. 0%) were also better in the OC pretreated group.

In a recent study, Lindheim *et al.* (80) also reported the effectiveness of this form of treatment. While in their study no significant improvements were noted on the stimulation characteristics of COH as measured by days of stimulation, number of ampules of gonadotropins used, peak E_2 and progesterone, number of retrieved oocytes compared to more standard stimulation protocols, and the pregnancy rate was significantly enhanced (30% vs. 6%). They proposed as potential mechanisms of action the production or alterations of local ovarian growth factors and/or changes in endometrial expression.

In summary, pretreatment with OC may represent an alternative protocol for poor responders, and may be worth attempting before directing patients to oocyte donation.

Natural Cycle for IVF

Stimulated cycles that result in only a few recruited follicles are similar to natural IVF cycles in that a single or a small cohort of dominant follicles is aspirated. Thus, in selected patients with a diminished ovarian reserve an attempt at IVF using a natural cycle may be offered with reasonable pregnancy rates. An early report of a successful pregnancy following natural cycle IVF was in a poor-responder patient.

Lindheim *et al.* (81) recently evaluated two groups of poor responders who were treated either by a long protocol using leuprolide acetate and hMG or by applying a natural cycle. There were no differences between the two groups regarding the number of embryos transferred and the pregnancy rates. A greater number of oocytes were retrieved using the stimulation protocol, but the fertilization and implantation rates were lower in this group, suggesting that the oocyte and embryo quality might be lower in stimulated cycles of poor responders than in natural cycles. Therefore, in cases of repeated failures with standard stimulation protocols or those reluctant to proceed with ovum donation employing natural cycles may be considered a last resort.

POSTOOCYTE RETRIEVAL TREATMENT BLASTOCYST TRANSFER

Blastocyst transfer may prove beneficial in several groups of patients: those with repeated implantation failure; those with uterine abnormalities that preclude multiple pregnancies, thus requiring more careful selection of the single embryo transferred; patients

suspected of defects in oocyte quality, thus requiring embryos to be assessed for a more extended period of in vitro development; patients needing embryo biopsy for genetic selection; and patients undergoing replacement of supernumerary embryos frozen at the blastocyst stage.

Animal experiments indicate a high implantation, pregnancy, and live birthrate when following blastocyst transfer (82). These observations indicate that human IVF-ET may be enhanced dramatically, if embryos were grown under improved conditions to the blastocyst stage before transfer.

Data to date on the replacement of human blastocysts on day 5 of development in culture indicate that such embryos have a high implantation rate. For good quality eight-cells and for expanded blastocysts, implantation rates of 18% and 35%, respectively, have been reported (82). Gardner *et al.* (83) evaluated the implantation rates achieved by the transfer of blastocysts on day 5 compared with embryos transferred on day 3. Implantation was significantly increased when embryos were transferred on day 5, compared with those transferred on day 3 (45.5% and 21%, respectively). The ongoing pregnancy rate for embryos transferred either on day 3 or on day 5 was equivalent, although significantly fewer embryos were transferred on day 5, thus minimizing the risk of multiple gestation (83).

Therefore, blastocyst culture and transfer in human IVF seems to provide potential advantages: synchronization of the embryo with endometrial development, leading to increased implantation rates, and selecting the most developmentally competent embryos for transfer, thereby avoiding transfer of embryos predestined to arrest. While these rationales also seem to be favored in the treatment of low responders, no study thus far has examined the value of blastocyst culture and transfer in this group of patients. Since the main problem of blastocyst transfer is the suboptimal culture conditions, implying that only a fraction of the embryos will reach the blastocyst stage, a great dilemma exists whether to apply this form of treatment to low responders, who a priori have a reduced number of embryos. Improved culture conditions leading to higher blastulation rates may provide the necessary tools for applying blastocyst culture and transfer in low responders.

ASSISTED HATCHING

Assisted zona hatching (AZH) has been applied in ART in order to promote hatching and subsequent embryo implantation by producing an artificial opening in the zona pellucida. In 1989, Cohen *et al.* (84) observed a higher implantation rate per embryo following partial zona dissection (PZD) and postulated that the opening made in the zona may aid the embryo in the hatching process. Since that report, many IVF programs have utilized assisted hatching to produce a small opening in the zona pellucida prior to embryo transfer in an effort to increase the rate of implantation. Initial results have demonstrated that assisted hatching may be beneficial in couples of advanced maternal age, elevated basal FSH concentration, repeated failed IVF or embryos with poor morphology due to increased zona thickness ($\geq$ 15 μm), high degree of fragmentation, and low developmental rate. Schoolcraft *et al.* (85) reported significantly increased pregnancy (64 vs. 19%) and implantation rates (33 vs. 6.5%) in poor-prognosis patients receiving assisted hatching compared with those who did not. In 1995, Stein *et al.* (86) evaluated assisted hatching using the PZD technique in 154 patients who had undergone at least three IVF treatment cycles yielding good-quality embryos but with no pregnancies initiated. The results demonstrated a significant increase in pregnancy rates only in patients aged $>$ 38 years (23.9%) compared with control patients (nonhatched) in the same age group (7%). There was no significant decrease in pregnancy rates demonstrated in patients aged $<$ 38 years. Other more recent reports have shown similar results, proposing a beneficial effect of AZH in poor prognosis patients (87).

In contrast to these reports, others have found no positive effect of AZH on IVF success rates in poor responders. In a recent prospective randomized study using the PZD technique, Hellebaut *et al.* (88) found no increase in pregnancy or implantation rates by the application of this procedure. In another study, Tucker *et al.* (89) found the clinical pregnancy rates to be significantly higher following AZH in patients aged $>$ 35 years (45.2%) compared with control patients (16.7%). However, the ongoing pregnancy and implantation rates were not significantly different among the two groups (89). Also, in a recent prospective, randomized, double-blind study, Lanzendorf *et al.* (90) reported no significant impact of AZH in their population studied.

Differences in methodology, study design, patient and embryo selection criteria, and low number of subjects may explain the diversity in the reported results and make the interpretation of the effects of AZH on IVF success rates difficult. It seems, however, that some groups of elderly poor-prognosis patients may

benefit from its application. In the near future, new improvements of laser zona dissection or transfer of thinned zona blastocysts may have a dramatic impact on the efforts directed toward improving embryo implantation.

CONVENTIONAL IVF VERSUS INTRACYTOPLASMIC SPERM INJECTION

Diminished number of retrieved oocytes has been associated with lower fertilization rates and IVF outcome. In addition, others reported that fertilization failure was present in about 16% of the IVF cycles and more frequent when fewer than three eggs were retrieved. In low responders, usually a small number of oocytes are retrieved after COH. Whether conventional IVF or intracytoplasmic sperm injection (ICSI) should be applied to this group in order to increase the fertilization rate remains still an open question.

In a prospective study of 96 low-responder patients, Moreno *et al.* (91) reported no differences in the fertilization rate, number of embryos transferred, and pregnancy and implantation rates, after ICSI or conventional IVF. They concluded that in cases of non-male infertility the technique of fertilization is not related to the reproductive outcome of low responders and that the routine use of ICSI is not indicated. Conversely, other recent reports have shown that ICSI can be used successfully on non-male factor couples to provide comparable or even higher fertilization rates and superior quality of embryos than those obtained after standard IVF (92). Due to the variability of the data and until further information is provided, it may be concluded that in low responders without male infertility, ICSI should be the method of choice in a second attempt when previous fertilization failure with conventional IVF has occurred.

OTHER POTENTIAL FORMS OF TREATMENT

Coculture in Assisted Reproduction

The marginal improvement in implantation rates achieved over the last years has enhanced researchers' efforts in evaluating other methods by which embryo development may be improved. As a result, a search for more optimal in vitro conditions was initiated, and significant progress has been achieved with the introduction of the coculture systems. This new technique also may be used as a diagnostic tool, as it can be applied to visualize embryo development until the time of transfer.

Various types of cells are being used in cocultures, and human tubal cells or bovine oviduct epithelial cells have been used in clinical programs. Coculture techniques using fetal bovine uterine fibroblasts or bovine oviductal epithelial cells (BOEC) have been shown to improve embryonic development prior to replacement in humans. Wiemer *et al.* (93) reported that the combination of coculture and selective assisted hatching provided a 45% ongoing pregnancy rate with a 23% implantation rate and concluded that certain patients' subgroups may benefit the most from coculture. These include patients who have previously failed IVF attempts, or have endocrine imbalances such as polycystic ovarian syndrome and elevated day 3 serum FSH levels. The benefit from applying coculture techniques in low responders currently is insufficiently investigated and its value unknown.

Cytoplasm and Nuclear Transfer in Human Oocytes

Waning oocyte quality seems to be the principal factor responsible for the age-related decline in female fertility. With increasing age, oocyte quality becomes impaired, leading to poor embryo quality and consequently low implantation rates and high pregnancy wastage. This deterioration in oocyte quality seems to result from cytoplasmic and nuclear alterations (4).

The role of the ooplasm in mammalian oocyte maturation and activation has been well documented but is far from complete understanding (94). An equally critical importance of ooplasmic factors is postulated for the continued development of the zygote, particularly during early cleavage, when transcription of the embryonic genome is minimal. Some nongenetic anomalies that originate in the ooplasm rather than in the embryonic genome may and often do interfere with normal development and as a consequence may threaten the viability of the embryo concerned. Keefe *et al.* (95) proposed that oocytes from older women were more likely to contain deleted mitochondrial DNA (mtDNA) than oocytes from younger women, and that these alterations could have adverse cellular effects by disrupting the normal electron and energy transport chain, leading to high levels of intracellular reactive oxygen species and cellular dysfunction.

Transfer of ooplasm from a donor oocyte obtained from a young patient may achieve restoration of normal developmental potential to eggs with ooplasm deficiencies. Patients who may benefit from this form of treatment are those that produce oocytes with an assumed normal nuclear genome but an ooplasm that is abnormal or deficient due to maternally mediated factors. Thus, the criteria used in selection of patients are crucially important, since ooplasm transfer clearly could not be expected to cure problems that are either causally unrelated to the constitution of the ooplasm of the mature egg or have become irreversible by the time the procedure is carried out. Cohen *et al.* (94) recently reported ooplasm transfer in seven couples (eight cycles) with multiple implantation failures by using either electrofusion of an ooplasmic donor fragment into each patient's egg (three cycles) or direct injection of ooplasm from a donor egg into each patient (five cycles). Normal fertilization and improvement in embryo morphology were significantly higher by applying the latter method, by which three pregnancies were achieved, leading to a normal pregnancy and delivery; a miscarriage; whereas a third pregnancy is still ongoing. Amniocentesis performed in the two pregnancies confirmed the nuclear characteristics matching those of the parents, thus excluding the possibility of transfer of nuclear material from the donor during the ooplasmic transfer. In addition, sensitive assays performed did not detect possible donor mtDNA in the fetus.

It also has been well established that in humans the number of chromosome abnormalities, especially aneuploidy, increases with age, affecting embryo development. Additionally, unfertilized oocytes from in vitro fertilization cycles have shown a high percentage of chromosomal perturbations. In a study, where 60 oocytes from 28 women between 27 and 41 years of age were analyzed, correlation was found between maternal age and aneuploidy in oocytes and between morphology and genetic balance in preimplantation embryos (96).

Nuclear transplantation from an older oocyte to a donor-enucleated cytoplasm also has been proposed in order to overcome any effects induced by age-related cytoplasmic alterations, and an initial report of germinal vesicle transfer has been described as successfully proceeding to extrusion of the first polar body. However, fertilization was not proved and progression to creation of a human embryo and to the establishment of pregnancy was not reported (96).

Cytoplasm and nuclear transfer in human oocytes may lead to an improvement of oocyte quality in low-response patients, particularly of older age, thus enhancing their implantation and pregnancy rates. However, these treatments currently are experimental in nature and their real efficacy is unclear. In addition, they raise a number of ethical issues, like the introduction of a completely new set of mtDNA from the donor to the recipient oocyte, which may lead to the creation of a three-parental individual. As a result of these unresolved issues and until further studies indicate the best approaches for their application, the demand for these new procedures may be overshadowed.

In Vitro Maturation of Immature Human Oocytes

Human oocyte maturation is considered as the reinitiation and completion of the first meiotic division from the germinal vesicle stage (prophase I) to metaphase II and the accompanying cytoplasmic maturation for fertilization and early embryonic development. In vitro maturation (IVM) of immature oocytes and the achievement of pregnancy potentially can be a major advance in ART as it obviates the need for the hyperstimulation of women, which is potentially hazardous and costly. The first pregnancy in humans from IVM oocytes was reported by Cha *et al.* (97), who aspirated oocytes in various stages of maturity from surgically removed ovaries, and cultured them for 32–48 h in two-culture systems, one containing fetal cord serum and another containing 50% mature follicular fluid. They reported 36% and 50% maturity rates and 32% and 81% fertilization rates in the two culture systems, respectively. A significant improvement to the application of this technique was later achieved by Trounson *et al.* (98), who reported that a reasonable oocyte recovery rate can be achieved by transvaginal ultrasound-guided follicle aspiration in small growing follicles ($\geq$ 3 mm diameter). However, their pregnancy and live birthrate was very low, reaching only 2% (98).

The nature of the culture medium seems to be the key factor contributing to the success of an IVM program, and thus many factors are being evaluated in an effort to increase the efficacy of this method. Currently, the present situation is that immature oocytes can be recovered efficiently from small follicles by transvaginal follicular aspiration. The oocytes show nuclear maturation changes consistent with ultrastructural and cytogenetic observations of oocytes matured in vivo and they fertilize and cleave as expected for in vivo matured oocytes recovered from IVF patients in whom ovulation was stimulated. However, in oocytes matured in vitro there still is a major problem of embry-

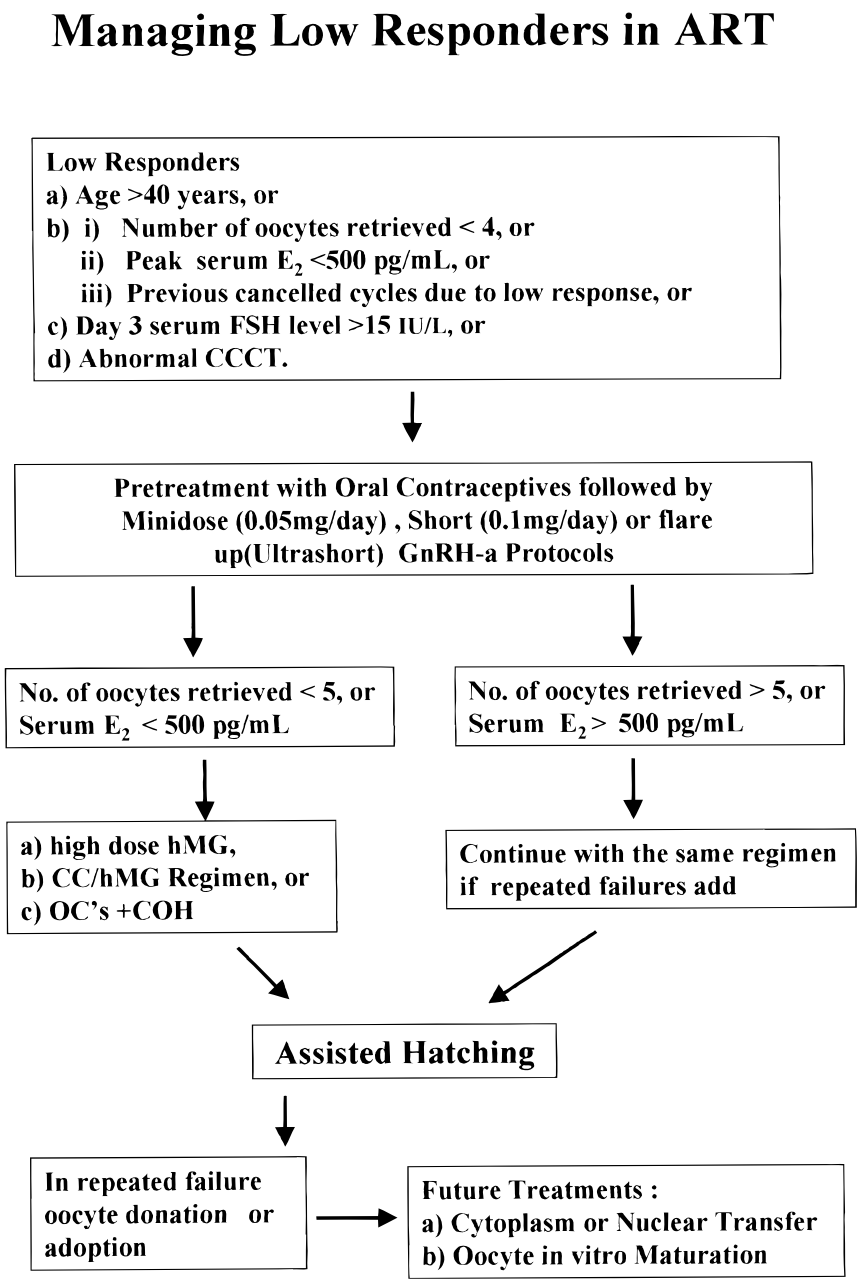

Fig. 1. Suggested flow chart for managing low responders in ART.

onic developmental competence, particularly cleavage and development beyond the four-cell stage (99).

It has been well known that after the age of 35 years, the endocrine system in women shows significant changes, with a rapid rise in serum FSH levels, and likely it can be expected that other regulatory systems change as well. Because of this change in the endocrine environment, follicular growth and oocyte maturation may be affected, and in turn this may affect oocyte quality (4). Evidence exists that an adequate estrogen intrafollicular milieu is important in the oocyte's acquisition of developmental competence and cytoplasmic maturation including activation, synthesis of the male pronucleus growth factor, and preimplantation development (100). Thus, it may be assumed that the low intrafollicular estrogen levels produced by poor responders may be associated with the low response to COH, and may affect at least in part an oocyte's

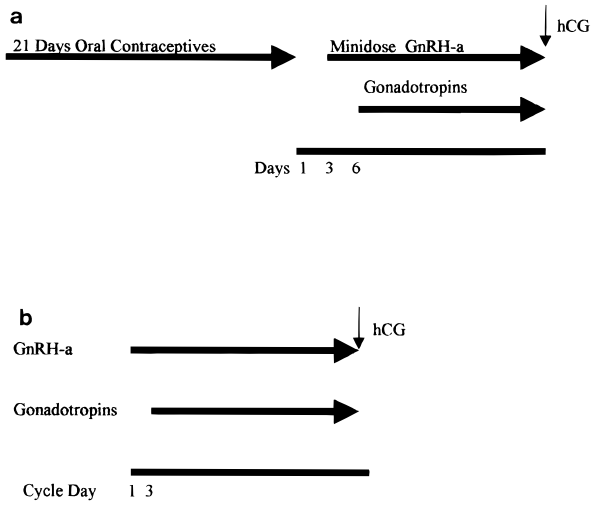

Fig. 2. Minidose GnRH-a protocol for low responders (a). Short GnRH-a protocol (b).

quality to develop into a viable pregnancy. Therefore, given the normal births reported from in vitro matured oocytes, it is likely that further research will result in improvements in in vitro oocyte and subsequent embryo developmental competence, thus overcoming a possible unfavorable hormonal intrafollicular milieu; in such cases, in vitro maturation of immature human oocytes will become an attractive alternative therapy in low response patients.

CONCLUSIONS

Two decades after the introduction of ART the evaluation and treatment of low responders remains a challenge and requires constant scrutiny and modification of currently used stimulation protocols. Based on the data currently available in the literature, it is possible to recommend guidelines for their management (Figs. 1 and 2). Importantly, improving oocyte retrieval, fertilization, and implantation and pregnancy rates in this group of patients should be achieved at a minimum increase in cost, duration of treatment, and patient's risks. Thus, a constant reevaluation of our current methods, in addition to employment of newer technologies, is warranted in order to enhance the chances for conception in low responders.

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