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Controlled ovarian hyperstimulation protocols for in vitro fertilization: two decades of experience after the birth of Elizabeth Carr

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Objective: To critically discuss the current protocols for the management of controlled ovarian hyperstimulation in assisted reproduction technology.
Design: Review of the literature and presentation of our experience.
Main Outcome Measure(s): Ovarian response (peak serum estrogen levels, number of oocytes retrieved, quality of oocytes and embryos) and pregnancy outcome (clinical, delivery, and multiple pregnancy rates).
Result(s): Controversies still exist regarding selection of gonadotropin preparation, choice of adjuvant therapy with GnRH analogues, and use of oral contraceptive pills. Patients identified as intermediate responders have an excellent outcome with adjuvant therapy with either a GnRH agonist (long protocol) or a GnRH antagonist, but tailoring of gonadotropin dose must be performed to achieve optimized results. High responders perform favorably with gentler gonadotropin stimulation that minimizes the occurrence of ovarian hyperstimulation syndrome. On the other hand, results in low responders remain suboptimal both in terms of ovarian response and oocyte/embryo quality in spite of a variety of stimulation regimens used.
Conclusion(s): Ovarian stimulation is a critical step in in vitro fertilization therapy. A variety of controlled ovarian hyperstimulation regimens are available and efficacious, but individualization of management is essential and depends on assessment of the ovarian reserve. Identification of the etiologies of poor ovarian response constitutes a formidable challenge facing reproductive endocrinologists. (Fertil Steril® 2005;84:555–69. ©2005 by American Society for Reproductive Medicine.)

Key Words: GnRH analogues, gonadotropins, IVF, ovarian reserve, ovarian stimulation

It has been 26 years since the birth of Louise Brown in Oldham, England, and 23 years since the birth of Elizabeth Carr in Norfolk, Connecticut, the first two IVF babies born in Europe and the United States, respectively. Current estimates indicate that the birth of over a million babies is the result of IVF worldwide. In vitro fertilization has become an established, highly efficient therapy for treating infertility, and childless couples with a variety of etiologic causes (female or male factor infertility, or combined) have benefitted from the increasing number of successes achieved by advanced reproductive technology (ART). Although the field continues to evolve at a very rapid pace, ART practitioners still face two major dilemmas: whether to continue to enhance conception rates, as approximately one in three embryo transfer cycles results in a live birth and approximately 20% to 30% of transferred embryos achieve implantation (1); and whether to decrease or eliminate multiple pregnancies that result in severe obstetric and perinatal complications and remarkably increase the cost of the treatment.

The major goals of IVF therapy are: [1] to obtain multiple fertilizable oocytes of good quality than can lead to diploid fertilization and early embryo development; [2] to establish a single, healthy (euploid) pregnancy following embryo transfer to the uterine cavity; and [3] to cryopreserve excess embryos of good quality to optimize the total reproductive potential (2, 3). Controlled ovarian hyperstimulation (COH) is therefore a principal step of IVF therapy. Ovarian stimulation regimens have undergone significant modifications; improved clinical experience and the availability of new hormonal preparations and adjuvant therapies have resulted in the development of new protocols. Here, we critically assess the current COH protocols via a selected review and

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discussion of the available literature, the controversies, and our clinical experience.

OVERALL MANAGEMENT OF COH FOR IVF

Four concepts are crucial for an optimized clinical management of COH for IVF: [1] the prospective identification of the ovarian response; [2] the individualization of COH treatment such that it is tailored to the recovery of a synchronous cohort of mature oocytes; [3] the prevention of potential complications; and [4] the optimization of the total reproductive potential by use of embryo cryopreservation technology.

Prospective Identification of the Ovarian Response: Assessment of the Ovarian Reserve

Jones et al. (4, 5) pioneered the use of gonadotropins for COH in IVF therapy. It was identified early that normally cycling, ovulatory women who underwent gonadotropin stimulation fell into one of three response categories: high, intermediate, or low responders. Furthermore, the individual's response was similar on subsequent stimulation cycles (5). The response category was based on the assessment of the resulting serum E_2 curve (E_2 pattern) and the consequent accompanying follicular response as monitored by ultrasonography. Moreover, the patient's response category and E_2 pattern were correlated with the capacity to achieve a pregnancy following IVF and embryo transfer. Two decades later, and after the introduction of improved gonadotropin preparations (urinary, highly purified, and later recombinant) and the use of adjuvant therapies such as GnRH agonists and antagonists, these concepts have remained intact and continue to guide clinical management.

Identifying a patient's ovarian response as high, intermediate, or low is essential to prospectively optimizing COH protocols and to diminishing the risk for complications such as cycle cancellation due to inadequate response or, conversely, development of ovarian hyperstimulation syndrome (OHSS). The ovarian response and the potential for conception of an IVF cycle can be determined with high accuracy by the assessment of the ovarian reserve.

Muasher et al. (6) first reported that the measurement of serum levels of FSH, LH, and E_2 on day 3 of the basal cycle was a predictor of COH response and IVF outcome. Subsequent studies established the clinical significance of defined thresholds for such hormones in addition to their relationship to the woman's age, thus further defining the concept of ovarian reserve (7–10).

Since then, many other tests have been introduced as candidates for the examination of the ovarian reserve. Such screening tests include the clomiphene citrate challenge test (CCCT) (11), GnRH test (6), GnRH agonist test (12), measurement of serum inhibin B (13) and antimüllerian hormone (14), and ultrasound examination of basal cycle ovarian volume, antral follicle count, and ovarian stromal blood flow (15–17).

Recently, a meta-analysis compared the positive and negative predictive value of basal serum FSH levels and the results of the CCCT for achieving a pregnancy in a population of infertility patients undergoing ovulation induction, intrauterine insemination therapy (IUI), or IVF (18). Results demonstrated that both tests have a high positive predictive value and the same ability to predict a clinical pregnancy. The study concluded that measuring basal cycle day-3 FSH levels is preferred over the CCCT because it is simpler and more cost effective. In our program, the determination of basal cycle day-3 FSH, LH, and E_2 levels is the preferred screening test for ovarian reserve in all patients.

Individualization of Treatment. We apply several general management concepts in our program. [1] We mimic physiology by administering a higher FSH dose in the early follicular phase (during the follicle recruitment phase). [2] We monitor serum E_2 levels and follicular development by ultrasound frequently, and continue stimulation using a step-down gonadotropin regimen, as selected codominant follicles achieve a relative degree of gonadotropin independence, and growth is maintained by the action of E_2 and other local ovarian factors (5, 19). [3] We administer the ovulation triggering dose of hCG when the leading follicles achieve $\geq$17 mm in diameter. [4] We perform a transvaginal ultrasound–guided harvest of the oocytes 34 to 35 hours after hCG administration.

Hodgen et al. (20) demonstrated in the cycling primate model that a step-down gonadotropin regimen resulted in more synchronous ovulations than a step-up protocol. Clinical experience has confirmed that this is the regimen of choice for IVF in ovulatory women. In anovulatory women, where the aim is to induce ovulation for timed intercourse or IUI therapy, starting with a low gonadotropin dose and progressing in a step-up fashion can lead to the achievement of a monofollicular ovulatory response. However, there are exceptions to the use of a step-down gonadotropin protocol in IVF. For example, atypical patients may not respond adequately to the initially chosen gonadotropin dose during the early follicular phase but will progress adequately after the gonadotropin amount is increased. We have observed such cases in association with an unexpectedly high degree of ovarian suppression in cycles down-regulated with a GnRH agonist or using oral contraceptives (OCs). In addition, patients in whom a GnRH antagonist was used may need gonadotropin support to be maintained at the onset of the antagonist administration.

Prevention of Potential Complications. Ovarian hyperstimulation syndrome (OHSS), a potentially lethal iatrogenic condition characterized by increased capillary permeability and a shift of intravascular fluid to extravascular spaces, can lead to hemoconcentration, coagulation disorders, and pulmonary embolism. Its incidence is 0.6% to 14% of ART cycles (21). The prospective identification of patients at risk

is essential to preventing or minimizing the development of the full syndrome.

Elevated E_2 levels at the time of hCG administration and release of certain cytokines are likely involved in the pathogenesis of OHSS (22). Navot et al. (23) identified the common characteristics of women prone to developing OHSS, which include younger age (<35 years), a lean body mass, and/or the presence of polycystic ovary syndrome (PCOS) or PCO-like ovaries. Careful consideration of the gonadotropin starting dose is crucial to prevent OHSS while aiming to recover an acceptable cohort of mature oocytes. Critical values of E_2 that trigger the syndrome are under debate and differ for ART (>4,000 pg/mL) and conventional ovulation induction methods (>1,700 pg/mL), possibly as a reflection of the patient's physiologic background and pretreatment ovarian suppression (23). Because hCG is the only known triggering factor for development of OHSS, the method of choice for reducing the risk in patients with E_2 above certain critical values (24) has been withdrawing gonadotropins and postponing hCG administration ("coasting"). The concept of withholding gonadotropins before hCG administration was initially introduced by Jones et al. (19, 25) in an effort to investigate the effect of the FSH/hMG/hCG interval on pregnancy outcome. A recent report from the Cochrane library indicated that there is insufficient evidence to determine whether coasting is an effective strategy for preventing OHSS (26). Nevertheless, it has been suggested that, when used to prevent OHSS, the coasting period should last <4 days to achieve optimal pregnancy results (27). Another alternative is the substitution of hCG by shorter acting compounds like a GnRH agonist (28). Cryopreservation of all embryos has also been proposed as an option, although controversial results have been published about its outcome and cost-efficiency (29).

Because multiple pregnancies have significantly higher perinatal mortality and morbidity than single births, multiple gestation pregnancy should be viewed as the other major complication of COH and ART. There is also a significant economic impact associated with multiple pregnancies (30). Although efforts to reduce multiple pregnancies have been introduced in the clinical IVF setting, its occurrence continues to be high (31). The incidence of multiple pregnancies can be reduced or eliminated through two strategies. [1] Decreasing the number of embryos transferred requires extensive work on improved culture conditions (32) and development of noninvasive methods to optimize the selection of the most viable embryos with highest implantation potential (33). Single embryo transfer in selected populations has resulted in acceptable pregnancy rates (34). Gardner et al. (35) noted that single blastocyst transfer in a highly selected population achieved excellent pregnancy rates. [2] Increasing the efficiency of implantation should follow the identification of embryonic and endometrial factors that establish and regulate the window of implantation (36).

Optimization of the Total Reproductive Potential by Use of Embryo Cryopreservation Technology. Embryo cryopreservation represents another remarkable achievement in ART. It provides multiple advantages including [1] the possibility of inseminating all retrieved mature oocytes without the need to discard any embryos; [2] limiting the number of embryos transferred to reduce the incidence of multiple pregnancy; [3] enhancing couples' chances of pregnancy by allowing multiple transfers originating from a single stimulated cycle (thereby optimizing their total reproductive potential); [4] aiding in the clinical management of OHSS; and [5] providing a valid, ethical means for evaluation of alternative research protocols (37).

We have performed embryo freezing at different phases of development, including pronuclear, cleaving, and blastocyst stages. Freezing-thawing procedures, cycle monitoring, and programmed protocols (estrogen/progesterone supplementation) have been published earlier (38–40). The delivery rate per transfer in our embryo cryopreservation program (initiated in 1986, in place to December 2003) in 2,793 cycles with embryo freezing and 10,573 thawed embryos was 22% (overall clinical pregnancy rate of 28%). The survival rate of embryos cryopreserved at various developmental stages was similar, pronuclear 69%, cleaving 68%, and blastocyst 62%, as was the implantation rate of 14%, 13%, and 19%, respectively (37). Similar results have been achieved when embryos were frozen in gonadotropin-stimulated cycles in combination with a GnRH agonist or GnRH antagonist (37, 41). We have observed no impact on outcome from the oocyte/embryo micromanipulation techniques and transfer protocols used (37). However, other investigators have demonstrated that zona pellucida manipulation has a negative impact on embryo cryosurvival and pregnancy rates (42).

The total reproductive potential is the true expression of pregnancy potential after a single cycle of stimulation (2, 3). It can be calculated using a model that includes the number of pregnancies arising from the transfer of fresh embryos alone and the number of pregnancies arising from subsequent transfer(s) of cryopreserved-thawed embryos. This is a truly patient-specific pregnancy that demonstrates the role of cryoaugmentation in the overall conception potential after one IVF cycle.

HORMONAL PREPARATIONS AND REGIMEN ALTERNATIVES

Gonadotropins are the fundamental agents used in ovulation stimulation. The combination of GnRH agonist pituitary suppression and exogenous gonadotropins in ART protocols has resulted in significant beneficial effects. These include: [1] improvement in the stimulation of follicular development and in the quality of developing oocytes; [2] prevention of a premature LH release; [3] decrease in cancellation rates; and [4] an overall improvement in the total reproductive potential (43, 44).

The first gonadotropin used was the urinary product hMG containing equal amounts of FSH and LH. Later on, purified and highly purified urinary FSH preparations came onto the market, the latter having <0.1 IU LH and <5% copurified proteins. Recombinant FSH, devoid of LH activity and with <1% copurified proteins, was introduced in the mid-1990s. Since then, many studies have compared the impact of recombinant and urinary FSH types containing variable amounts of LH activity in COH and demonstrated diverging results.

The ideal way to compare clinical results after the use of urinary and recombinant FSH preparations is to perform prospective, randomized, multicenter studies in homogeneous groups of patients. There are several studies in the literature fulfilling those criteria (45–54). Out et al. (45) compared recombinant FSH with urinary FSH and concluded that the recombinant hormone was more effective at inducing multifollicular development and in achieving an ongoing pregnancy than the urinary preparation. Bergh et al. (46) found that recombinant FSH was more effective than highly purified urinary FSH at inducing multiple follicular development. Frydman et al. (49) reported a higher mean number of oocytes retrieved and lower dose of FSH used with recombinant preparations. On the other hand, the pregnancy rates were comparable with both types of gonadotropins used. Ng et al. (51) did not find any difference in pregnany or implantation rates between recombinant FSH and hMG groups. Gerli et al. (54) evaluated the efficiency and cost-effectiveness of recombinant and urinary FSH in IVF cycles and reported no difference in terms of follicular development, length of stimulation, or pregnancy and delivery rates.

There are also limited data related to the use of recombinant FSH in poor responders. Raga et al. (55) and De Placido et al. (56) reported improved results with recombinant FSH when compared with urinary FSH for the number of oocytes retrieved and pregnancy rates in poor responders. Gleicher et al. (57) discussed an age-specific difference in response to recombinant and urinary gonadotropins and proposed that very young (≤34 years) or very old (≥41 years) women with poor response benefited more from recombinant FSH.

In spite of the results of such prospective randomized studies, a difference between recombinant and urinary FSH has not been completely demonstrated, especially for ongoing pregnancy rates. Two meta-analyses showed slightly increased pregnancy rates with recombinant FSH in IVF cycles compared with urinary products (58, 59). On the other hand, Agarwal et al. (60) could not find any difference in their meta-analysis (60). Recently, van Wely et al. (61) reported higher clinical pregnancy rates with the use of hMG compared with recombinant FSH. These conflicting results are probably related to the main shortcomings of meta-analysis, which allows postrandomization manipulations and underestimation of some of the covariables in the included studies, such as gonadotropin dose, type of urinary FSH, criteria for hCG administration, day of embryo transfer, and/or endometrial thickness at the time of embryo transfer (62).

After identification of LH effects during the follicular phase of stimulated cycles (63, 64) and the presentation of the "LH ceiling effect hypothesis" by Hiller (65), new clinical studies emerged reevaluating the effect of LH on ovulation stimulation protocols in ovulatory and anovulatory women. The role of exogenous LH during ovarian stimulation in normogonadotropic women down-regulated or not with a GnRH agonist has been a matter of considerable debate. Controversy exists as to the addition of LH to recombinant FSH to optimize COH results (66–69). The need for LH supplementation is not questioned in the case of patients with hypogonadotropic hypogonadism undergoing ovulation induction. Treatment of such profoundly hypogonadotropic women with FSH-only regimens results in reduced folliculogenesis and reduced levels of serum estrogen. In such cases, the addition of LH enhances E_2 secretion, follicle development, and the chances of achieving term pregnancy (70).

In normogonadotropic women, on the other hand, the administration of a GnRH agonist results in a variable level of suppression of pituitary gonadotropin secretion that depends on the type, dose, and mode of administration of the analogue. It is estimated that the amount of residual endogenous LH present during GnRH agonist pituitary suppression may be sufficient in most clinical cases to achieve adequate follicular maturation during ovarian stimulation with pure FSH. Nevertheless, it is also possible that in some cases GnRH down-regulation may result in profound suppression of LH concentrations with an adverse effect on steroidogenesis or oocyte quality, thus having an impact on IVF outcome. Such cases might benefit from preparations containing LH. "Threshold" and "ceiling" levels for LH (therapeutic window) have been proposed below which E_2 production is not adequate and above which LH may be detrimental to follicular development (69). Tesarik and Mendoza (71) have presented strong evidence in favor of this hypothesis.

In a retrospective study, we recently demonstrated that the measured suppressed serum LH levels during the early and late follicular phases in women <40 years of age with normal ovarian function desensitized with GnRH agonist and treated with recombinant FSH were not predictive of ovarian response to stimulation or pregnancy (72). The concentrations of residual endogenous LH present after GnRH agonist pituitary suppression with the regimen we employed certainly appeared to be sufficient to achieve adequate follicular maturation during ovarian stimulation with recombinant FSH. Therefore, our data do not support the need for exogenous LH supplementation in this clinical scenario.

In our early experience with the purified gonadotropin preparations, we demonstrated an equivalent ovarian response and IVF outcome when comparing urinary FSH

and hMG used in combination with a GnRH agonist (73). Furthermore, we reported that an improved oocyte quality was obtained with urinary FSH alone compared with urinary FSH/hMG combinations (74). More recently, Balasch et al. (75) reported on adverse effects of hMG on oocyte and embryo quality in agonist-suppressed cycles stimulated with recombinant FSH. We have also retrospectively compared recombinant FSH and urinary FSH preparations (recombinant FSH vs. urinary FSH and highly purified urinary FSH) in their capacity to induce multifollicular development and in their impact on IVF/intracytoplasmic sperm injection outcome in different categories of ovarian responders (76). Results demonstrated that, using the long GnRH agonist protocol or luteal suppression, all FSH preparations were equally effective for inducing multifollicular development.

Consequently, we agree with Shoham (69) that most patients treated with pituitary down-regulation will respond adequately to recombinant FSH alone and that probably a small group of patients who exhibit profound pituitary suppression benefit from exogenous LH support. In such cases, addition of LH by recombinant LH, micro-doses of urinary hCG, or hMG appear to provide enough support for an adequate folliculogenesis (66).

The use of recombinant LH was introduced in women with hypogonadotrophic hypogonadism in combination with recombinant FSH to facilitate folliculogenesis (70, 77). Laml et al. (78) reported the beneficial effect of recombinant LH administration in women with low LH concentration after down-regulation in the cancelled cycle and low response to the GnRH-agonist long protocol with FSH. Such results were supported by the study of De Placido et al. (79). On the contrary, in a prospective randomized study with a small number of patients, Balasch et al. (80) found a detrimental effect of recombinant LH addition on number of metaphase II oocytes retrieved and fertilization rate in patients treated with conventional IVF. Further studies are needed to investigate the value of recombinant LH administration during the late stages of follicular maturation.

The relatively recent incorporation of GnRH antagonists as adjuvants to COH has provided an efficient alternative for the management of COH. Gonadotropin-releasing hormone antagonists cause a prompt decrease in gonadotropin secretion, and they are now widely used to prevent a premature LH surge (81). They can be used either in a single high-dose or in multiple low-dose regimens. These compounds are started either on day 6 or day 7 of gonadotropin stimulation (fixed regimen) or on the day when the dominant follicle reaches a certain diameter (typically >14 mm) (flexible regimen). Although Kolibianakis et al. (82) reported higher implantation and pregnancy rates with a fixed regimen, two recently published prospective and randomized studies found similar results when comparing both protocols for the number of retrieved oocytes and clinical pregnancy rates (83, 84).

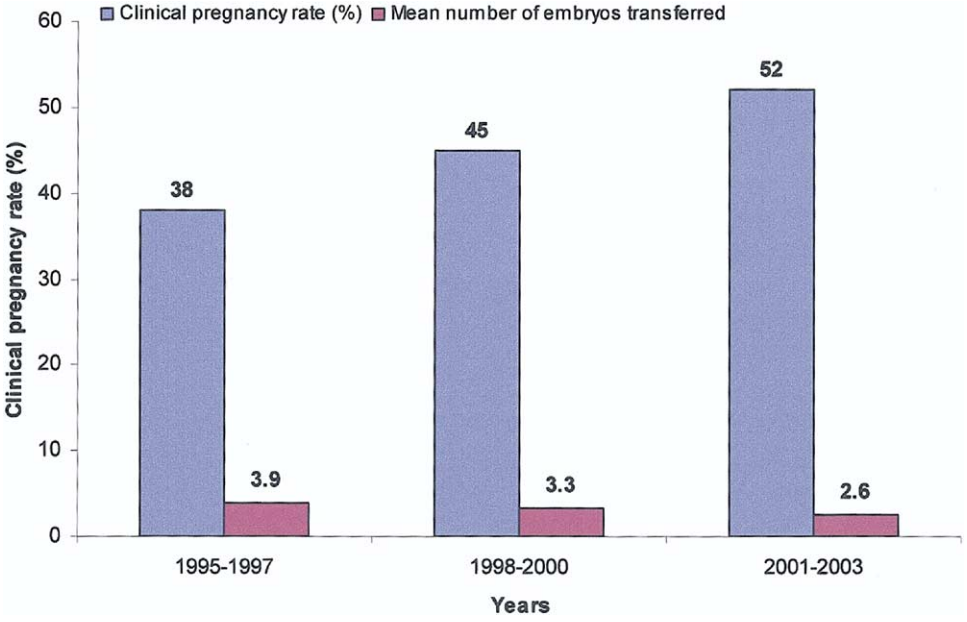
Overall, the benefits of GnRH antagonists appear to include simplification of the protocol and reduced use of gonadotropin ampules. Multicenter, randomized, prospective studies comparing GnRH agonist and antagonist treatment found that the length of stimulation and number of gonadotropin ampules administered were lower in the antagonist group. Although patients receiving the GnRH antagonist had the lower number of oocytes and embryos, the percentage of mature oocytes and fertilization rates were similar in both groups (85–87). However, in a meta-analysis composed of five randomized controlled studies comparing GnRH agonist protocols with fixed regimen GnRH antagonist protocols, Al-Inany and Aboulghar (88) reported significantly lower clinical pregnancy rates in the GnRH antagonist group in spite of the transfer of an equivalent number of good quality embryos in both groups. Moreover, increasing the dose of FSH on the day of GnRH antagonist administration in the flexible regimen did not result in an improvement in the number of the oocytes retrieved or in the pregnancy rates (89). Retrieval of a lower number of oocytes with use of GnRH antagonists could compromise the total reproductive potential, as fewer embryos might be available for cryopreservation. Further studies are needed to clarify this point.

Improved pregnancy rates were reported in poor responders treated with a GnRH antagonist compared with a GnRH agonist (long protocol) treatment (90). In addition, in a prospectively designed study, Akman et al. (91) concluded that the addition of a GnRH antagonist might benefit poor responders. Fasouliotis et al. (92) performed a retrospective study comparing a GnRH antagonist with the micro-flare GnRH agonist in poor responders and concluded that the GnRH antagonist resulted in a higher pregnancy rate. Dragisic et al. (93) reported lower cancellation rates and improved IVF outcome via a combination of estrogen therapy and GnRH antagonist started in the midluteal phase the preceding menstrual cycle. This is an interesting maneuver to suppress early follicular recruitment that typically occurs in the late luteal phase in patients with premenopausal status. Nevertheless, larger prospective randomized studies are needed to verify those results.

It has also been shown that the use of an oral contraceptive (OC) in the previous cycle may increase pregnancy rates in IVF (94). Because OCs have a putative role in enhancement of estrogen receptor sensitization due to their estrogen content, in addition to exerting pituitary suppression, they have been used in combination with GnRH agonists (95). Biljan et al. (96) reported that pituitary suppression with OC and a GnRH agonist was superior to GnRH agonist alone regarding pregnancy outcome. Because of these promising effects, OCs have also been used in poor responders. However, there is only one retrospective study evaluating the actual contribution of OC in this group of patients. Lindheim et al. (97) found higher pregnancy rates with OC alone compared with GnRH agonist–treated cycles (both long and flare protocols). They concluded that the good outcome associated with OC

FIGURE 1

Clinical pregnancy rates per transfer and average number of embryos transferred per cycle from 1995 to 2003 in our program (3-year intervals).



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pretreatment might reflect production or alterations of local ovarian growth factors and/or changes at the endometrial level.

Although urinary hCG has been the traditional compound used to trigger final follicular/oocyte maturation, novel alternatives have been proposed. A newly developed recombinant hCG (250 μg) was compared with urinary hCG (5,000 IU) in two prospective, double-blind, randomized studies (98, 99). The studies did not show significant differences between groups in the number of mature oocytes recovered, number of fertilized oocytes, number of embryos obtained, pregnancy rates, or incidence of OHSS. Serum hCG and progesterone concentrations were found to be higher in the post-hCG period in the recombinant group.

Recently, the European Recombinant LH Study Group published a prospective, dose-defining, comparative study in patients undergoing IVF (100). All patients underwent a long GnRH agonist protocol for pituitary suppression. After ovarian stimulation with recombinant FSH, different single or multiple doses of recombinant LH were applied and compared with 5,000 IU urinary hCG in inducing final follicular maturation and luteinization. The investigators found significantly lower OHSS in the single-dose recombinant LH protocol. There were no differences in the number of oocytes retrieved per follicle >10 mm, number of embryos, or pregnancy rates. More randomized studies and experience are required to clarify potential advantages of recombinant LH over conventionally used urinary hCG.

MANAGEMENT OF COH PROTOCOLS ACCORDING TO THE INDIVIDUAL'S PREDICTED OVARIAN RESPONSE

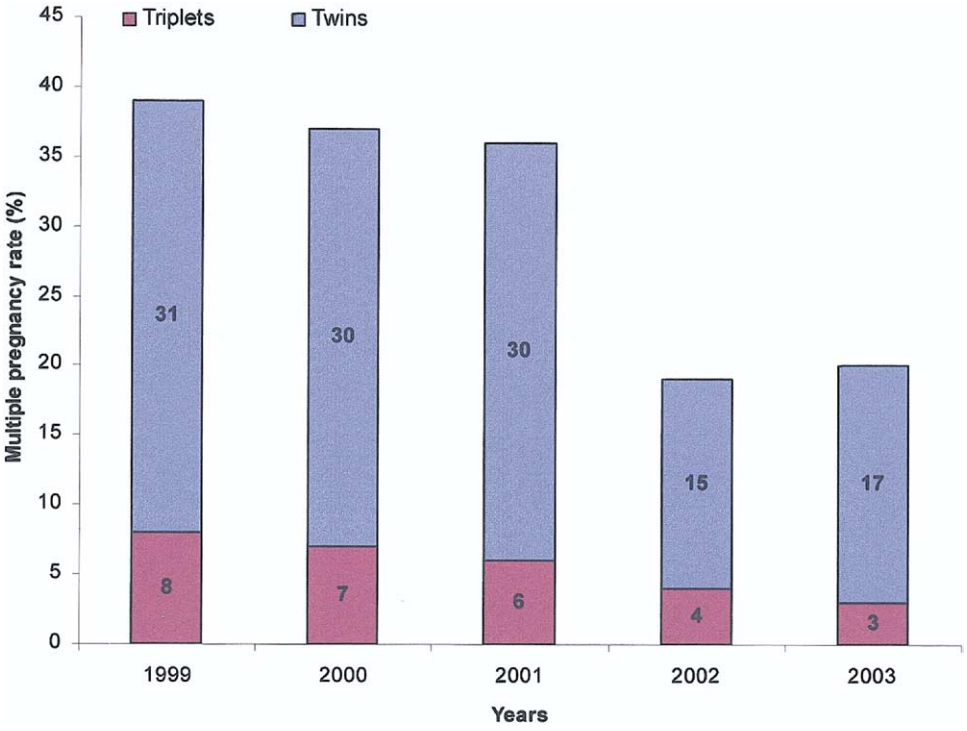
Since its inception in 1981 and up to December 2003, our ART program has performed 12,532 IVF cycles; 3,010 babies have been born, with 73% of deliveries being singletons, 23% twins, 3% triplets, and <1% quadruplets, for an overall multiple pregnancy rate per delivery of 27%. This delivery rate excludes multiple pregnancies that miscarried and/or were subjected to selective reduction.

Throughout the world, IVF results have improved. An overall assessment of our own results in intermediate and high responders for the years 1995 through 2003 shows a steady increase in clinical pregnancies (Fig. 1). This is probably multifactorial and the result of improved hormonal preparations, optimized culture conditions, and modifications in embryo transfer technique (such as the use of a soft catheter and transabdominal ultrasound guidance). It is important to note that the increased pregnancy rates have been achieved while decreasing the number of embryos transferred (from an average of 3.8 to 2.6 embryos) (see Fig. 1). This has followed an effort to reduce multiple pregnancies.

In this section, we present COH protocols and IVF results from the last 5-year period in our program (1999–2003). During that period, a total of 805 couples underwent 993 IVF cycles; the patients presented in the following infertility categories: tubal (40%), male factor (32%), endometriosis (15%), idiopathic (5%), ovulatory (3%), and other (5%). Serum gonadotropins and E_2 levels were measured with a

FIGURE 2

Incidence of multiple pregnancies during the last 5-year period (1999 to 2003) in our program. An overall decrease in multiple pregnancies ($P<.02$) was associated with a decreased occurrence of twins ($P<.05$) and triplets (not statistically significant).



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microparticle enzyme immunoassay (101). Leuprolide acetate was the GnRH agonist, and ganirelix was the GnRH antagonist used throughout the period. Recombinant FSH was the preparation of choice. However, and according to patients' history and other characteristics as well as monitoring of the ovarian response, hMG was incorporated into some protocols. Human chorionic gonadotropin (hCG 10,000 IU IM) or recombinant hCG (0.250 mg SC) were used to trigger ovulation, and supplementation of the luteal phase was performed with vaginal micronized progesterone (600 mg daily). For the most part, embryo transfers were performed on day 3 under transabdominal ultrasound guidance (102). Results are presented as mean ± standard deviation.

Overall, the mean number of mature oocytes retrieved (metaphase II oocytes) was 10 to 5, and the mean number of embryos transferred was 3 to 0.9. The overall clinical pregnancy rate per transfer was 41%, and the delivery rate was 31% (implantation rate of 19% and miscarriage rate of 21%). The overall incidence of multiple pregnancies was 35%. This is still a high incidence, although, of all conceptions, 29% were twin pregnancies and 6% were triplets.

The implemented change in embryo transfer policy mentioned above has lead to a moderate decrease in the incidence of multiple conceptions. Figure 2 presents the incidence of multiple pregnancies (clinical pregnancies defined as conception confirmed by ultrasound visualization of gestational sac) throughout the period under scrutiny. As can be observed, the total ($P<.02$), twin ($P<.05$) and triplet (lower but not significant) multiple pregnancy rates have steadily declined through the period under analysis. Such decline was parallel to the reduction in the number of embryos transferred (see Figs. 1, 2). Nevertheless, substantial work is needed to further decrease or eliminate the occurrence of this complication.

Management of Intermediate Responders

Intermediate responders were prospectively identified as those patients having a normal FSH/LH ratio and a normal ovarian volume with an adequate number of antral follicles. Such women were stimulated with a combination of a GnRH agonist (long protocol, luteal suppression) and recombinant FSH (n = 372 cycles). Subcutaneous leuprolide acetate was started on day 21 of the preceding luteal phase at the dose of 0.5 mg/d and decreased to 0.25 mg/d at menses. Recombinant FSH was initiated on day 3 of the menstrual cycle (after GnRH agonist down-regulation) at the daily starting dose of 225–300 IU/d. The dose was then adjusted in an individu-

TABLE 1

In vitro fertilization results in intermediate and high responders during the last 5-year period (1999–2003).

	Intermediate responders	High responders
No. of cycles	417	108
Age (y)	32 ± 3	31 ± 2
Basal day 3 FSH (IU/L)	5.8 ± 3.3	6.9 ± 3.4
Basal day 3 LH (IU/L)[a]	5.3 ± 3.7	8.9 ± 4.0
Basal day E_2 (pg/mL)	50 ± 20	48 ± 21
Peak serum E_2 (pg/mL)	2,809 ± 1,400	3,141 ± 1,800
Day of hCG[a]	12.1 ± 1.6	13.2 ± 1.7
No. of ampules FSH	27 ± 7	27 ± 8
No. of ampules hMG	11 ± 6 (n = 61)	9 ± 6 (n = 7)
No. of mature oocytes	12 ± 6	13 ± 7
Fertilization rate (%)	83	81
No. of embryos transferred	2.9 ± 0.7	2.6 ± 0.6
Clinical pregnancy rate/transfer (%)	53	61
Delivery rate/transfer (%)	43	39
Miscarriage rate (%)	18	35
Implantation rate (%)[b]	25	35
Percentage of cycles with freezing	48	51
Cryo clinical pregnancy rate/transfer (%)	43	44
Cryo implantation rate (%)[c]	19	26

[a] $P<.001$.
[b] $P<.05$.
[c] $P<.01$.

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alized fashion using a step-down protocol while monitoring serum E_2 levels and follicular development and growth by transvaginal ultrasonography.

A group of intermediate responders was allocated to a different protocol (nonrandomized) using a GnRH antagonist in a flexible regimen. In such cases, patients were pretreated with an OC for 3 weeks (a combination of 0.03 mg of ethinyl estradiol and 0.15 mg of desogestrel) from day 1 to 21 of the preceding cycle followed by menses and initiation of recombinant FSH on day 3 at the dose of 225–300 IU/d. The GnRH antagonist was commenced when the leading follicle reached 14 mm in diameter (0.250 mg/d). The gonadotropin dose was maintained (and not stepped down) during the days of GnRH antagonist administration. Results in this subgroup of patients (n = 45 cycles) in terms of COH response and pregnancy outcome were similar to the GnRH agonist-treated patients and were therefore combined for data presentation.

Table 1 presents the results of all intermediate responders (n = 417 cycles). Patients achieved a moderate average peak level of serum E_2 of 2,809 pg/mL and used an average of 27 ampules of FSH (75 IU per ampule). The clinical pregnancy rate per transfer was 53%, and the implantation rate was 25%. Forty-eight percent of these cycles had extra embryos that were cryopreserved, and the clinical pregnancy rate in thawed cycles was 43%. The group of intermediate responders demonstrated a total reproductive potential of 65%.

Management of High Responders

High responders were prospectively identified as those patients with PCOS or PCO-like features with either a high LH/FSH ratio or a multifollicular ovarian appearance. They were allocated to a GnRH agonist down-regulation protocol (luteal suppression) and recombinant FSH. Subcutaneous leuprolide acetate was started on day 21 of the preceding luteal phase at the dose of 0.5 mg/d and decreased to 0.25 mg/d at menses. Recombinant FSH was initiated on day 3 of the menstrual cycle (after GnRH-agonist down-regulation) at

the daily starting dose of 150 IU/d. The dose was then adjusted in an individualized fashion using a step-down protocol while monitoring serum E_2 levels and follicular development and growth by transvaginal ultrasonography. In the more severe cases of PCOS, an overlapping OC/GnRH agonist protocol was used before gonadotropin initiation. The OC was administered on day 1 of the preceding cycle up to day 7, and overlapped with the GnRH agonist for a week (days 7 to 14); then the OC was stopped while the GnRH agonist continued (103).

The results of the patients that were prospectively categorized as high responders (n = 108 cycles) are shown in Table 1. Compared with the intermediate responders and as expected, the group of high responders showed an elevated and statistically significant higher LH/FSH ratio ($P<.01$). The peak serum E_2 levels and number of mature oocytes were slightly higher (although not statistically significant) in the high responders. This is a reflection of management aimed to avoid OHSS. There was a trend for a higher clinical pregnancy, and the implantation rate was statistically significantly higher ($P<.05$) in the high responder group compared with the intermediate responders. However, there was a higher miscarriage rate (although not statistically significant, and probably due to the small sample size) in the high responder group, leading to similar delivery rates between the two groups.

It has been the policy of our program to aim at preventing the potentially severe complication of OHSS by adequately identifying patients at risk and by allocating them to this "gentler" stimulation regimen. In spite of this, a subgroup of patients may still develop an excessive number of follicles with achievement of very high E_2 levels. In this situation, one or a combination of the following measures were taken: [1] withdraw of gonadotropin support or "coasting" (implemented when E_2 levels achieved critical levels, typically around 5,000 pg/mL); [2] decrease the ovulatory dose of hCG in half (23); and [3] cryopreservation of all embryos to avoid transfer during the stimulated cycle. In the period under study, the incidence of OHSS for all cycles was only 2%, the majority of cases being mild or moderate. These patients were managed in an ambulatory fashion with fluid intake recommendation and early paracentesis as appropriate. There was only one case of severe hyperstimulation that required hospitalization, intravenous fluid replacement, repeated paracentesis, and heparin administration due to pulmonary embolism. The patient fully recovered and delivered healthy twins. In our experience, cryopreservation of all embryos at the pronuclear stage in such cases does not eliminate the syndrome, but the chances of pregnancy are excellent in subsequent transfer cycles of frozen-thawed embryos (40).

A few studies have shown an association between high serum LH levels as seen in women with PCOS and an increased incidence of miscarriage (104). Stanger and Yovich (105) found a significant reduction in the fertilization rate of mature oocytes in patients whose basal serum LH values were high. We have recently presented evidence that healthy, ovulatory oocyte donors with a relatively high serum level of LH on day 3 of the basal cycle of more than 9 UI/L had a significantly higher risk of miscarriage in the recipient population (likelihood ratio 4.3). This pointed to a negative effect on oocyte quality due to chronic elevation of serum LH levels (101).

Stadtmauer et al. (106) reviewed the data supporting the effect of metformin on improving hyperandrogenemia and hyperinsulinemia in PCOS patients. Published data support that metformin can benefit PCOS patients undergoing gonadotropin therapy and IVF as well as ovulation induction. It has been shown that metformin facilitated FSH stimulation and led to more mature oocytes and higher quality embryos and improved pregnancy rates in IVF (107). A recent randomized, prospective study of metformin treatment before IVF/intracytoplasmic sperm injection in PCOS showed no improvement of clinical outcomes except for the subgroup of patients with normal weight where pregnancy rate was higher (108).

Management of Low Responders

The definition of poor response has differed widely in the literature and has included the woman's age, basal hormonal status (high FSH), previous cancellation, and/or a poor response in a previous cycle with more than four oocytes retrieved and/or a peak serum E_2 level <500 pg/mL (90, 109–111). Notwithstanding definition inconsistencies, this group of women has the poorest prognosis for COH results and IVF pregnancy outcome.

Multiple COH strategies have been implemented in this group of challenging patients. They have included: high FSH doses (112), clomiphene citrate and hMG (113), micro-flare with a GnRH agonist (114), flare GnRH agonist protocol (115–117), stop-GnRH agonist protocol (118), growth hormone (119), and use of GnRH antagonists (91). Reassessment of natural cycle IVF has been suggested as an alternative approach in this group of patients (120). This variety of protocols reflects a high within-group variability, a probably multifactorial origin but more importantly, an overall compromised outcome.

In our program, low responders were prospectively identified as patients with one or more of the following characteristics: high basal cycle day 3 FSH (≥10 mIU/mL) or E_2 levels (≥90 pg/mL), advanced age (≥37 years), and/or low ovarian volume and/or a reduced number of antral follicles. If the patient had a previous IVF attempt, allocation to this response group required a previous cycle with a peak serum E_2 of <900 pg/mL, and/or retrieval of less than five mature oocytes, and/or previous cancellation due to inadequate folliculogenesis (fewer than four dominant follicles after 6 days of gonadotropin stimulation). Using such definition, low responders constitute a very large proportion of our patient

population. In fact, 47% of cycles performed during 1999 to 2003 were low responders. It is important to consider that there are poor responders <37 years of age and even with a normal basal FSH and E_2 levels; some of these patients can be identified upon basal ultrasound assessment of ovarian volume and antral follicle count. Other patients can be identified by an abnormal response to the CCCT (121) and occasionally by a high basal day 3 FSH/LH ratio (122, 123). A limited ovarian reserve with fewer developing follicles and low E_2 production characterize the response to COH. Occasionally, a low ovarian reserve is secondary to age, previous ovarian surgery, severe endometriosis, and/or pelvic adhesive disease, or ovarian failure due to an immunologic origin, but in others it remains unexplained.

More importantly, oocyte quality may be compromised in this group of women. Advanced maternal age is clearly associated with oocyte aneuploidy (124). We have described anomalies of the zona pellucida in oocytes recovered from poor responders (125). Such anomalies were characterized by an abnormal protein backbone as measured with specific anti-ZP3 antibodies. Other cytoplasmic abnormalities can also be present. Although the true pathogenesis of the poor ovarian response is unknown in a proportion of cases, it appears that this is a heterogeneous group of patients with intrinsic and/or stimulation-derived defects leading to a defined poor responder phenotype.

During the period under study, we have stimulated low responders (n = 468 cycles) with one of three protocols. Patients were allocated to treatment in a nonrandomized fashion and according to the treating physician's preference for a given protocol. One of the regimens consisted of the stop GnRH agonist protocol (118). Here, the luteal phase-initiated GnRH agonist (0.5 mg/d) was terminated at the onset of menses, and recombinant FSH was initiated at the high dose of 450 IU/d. Other low responders were stimulated with a high FSH dose with either a micro-flare GnRH agonist or a GnRH antagonist protocol. For both protocols, patients were pretreated with OCs for 3 weeks and gonadotropins were initiated after 4 days of the last active pill. In the micro-flare regimen, the GnRH agonist was started on day 2 at the dose of 40 μg twice daily and continued until hCG administration. Recombinant FSH was initiated on day 5 at the dose of 450 IU daily. For the GnRH antagonist flexible regimen, recombinant FSH (also 450 IU daily) was started on day 3 and the antagonist commenced when the leading follicle was 14 mm in diameter. In many of these patients, hMG was added to the stimulation. In some cases, the total gonadotropin dose of 450 IU was divided into 300 IU of recombinant FSH and 150 IU of hMG; in others, hMG (75–150 IU) was added at the time of GnRH antagonist initiation.

Table 2 presents the results of all three protocols. As can be observed, the stop GnRH agonist protocol resulted in a discreetly higher number of mature oocytes retrieved (although not statistically significantly different from the other two protocols) and a statistically significant higher delivery rate. This may reflect the nonrandomized allocation of patients with slightly better prognosis to this particular protocol (i.e., lower proportion of patients >40 years and lower number of previous canceled cycles compared with the other two regimens) rather than to a more suitable regimen for poor responders. The micro-flare and GnRH antagonist treatments resulted in a lower number of recombinant FSH ampules being used, but hMG was added in approximately 50% of those cycles. The delivery rates ranged from 12% to 27% and only 18% to 37% of the cycles resulted in extra embryos that were cryopreserved. The actual total reproductive potential for all low responders ranged from 23% to 40%. As expected, the total reproductive potential of low responders was statistically significantly lower ($P<.01$) than the total reproductive potential of intermediate and high responders.

To better compare the micro-flare GnRH agonist and the GnRH antagonist protocols, we analyzed a subset of patients (n = 52 and n = 64 patients, respectively) undergoing their first IVF stimulation attempt who were matched by age and basal hormonal levels. In this retrospective comparison of more homogeneous groups, we did not observe any difference in COH response (not shown) or pregnancy success (clinical pregnancy rate/transfer of 20% and 22%, for the micro-flare and GnRH antagonist protocols, respectively).

We also retrospectively analyzed the impact of pretreatment with OCs on the outcome of poor responders stimulated with a GnRH agonist micro-flare protocol. A total of 84 patients were studied (nonrandomized), 71 receiving OCs and 23 not receiving OC treatment before the micro-flare initiation. There were no statistically significant differences in COH results or pregnancy outcome, except for a slight but statistically significant higher percentage of metaphase II oocytes recovered in the OC group. We were interested in the comparison of basal FSH, LH, and E_2 levels before and after OC treatment (on the previous basal cycle day 3 and on day 3 after OC-induced menses). We found a variable response; some patients were suppressed after OCs as determined by low gonadotropin and E_2 levels, but others showed a paradoxically elevated FSH. However, using logistic regression analysis, we could not demonstrate any statistical correlation between FSH changes (ΔFSH) and the parameters of COH response, pregnancy, implantation, or miscarriage rates (not shown).

CONCLUSIONS AND FUTURE PROSPECTS

We have reviewed the current status of COH protocols and presented our recent experience and results obtained during the last 5-year period. We critically analyzed the extensive literature within this context. We presented patients' management and outcome according to the prospectively identified ovarian response. As a consequence of the diagnostic screening, each patient was allocated to a COH protocol in a predetermined fashion, and their management was tailored individually. The use of recombinant FSH in combination

TABLE 2

In vitro fertilization results in low responders during the last 5-year period (1999–2003).

Type of protocol	Stop GnRH agonist	Micro-flare	GnRH antagonist
No. of cycles	245	85	138
Age (y)	37.1 ± 3.6	37.9 ± 3.9	35.4 ± 4.7
Basal day 3 FSH (IU/L)	7.5 ± 2.6	7.8 ± 3.1	7.4 ± 3.1
Basal day 3 LH (IU/L)	4.8 ± 2.1	4.4 ± 2.3	4.5 ± 2.2
Basal day 3 E_2 (pg/mL)	50 ± 21	53 ± 21	49 ± 20
Peak serum E_2 (pg/mL)	1,830 ± 1,100	2,152 ± 1,300	1,733 ± 1,000
Day of hCG	12.3 ± 1.0	12.5 ± 1.4	11.9 ± 1.4
No. of ampules FSH	48 ± 11	39 ± 11	33 ± 17
No. of ampules hMG	15 ± 9 (n = 64)	18 ± 9 (n = 44)	14 ± 8 (n = 82)
No. of mature oocytes	9.5 ± 5	6.9 ± 3.5	7.8 ± 5.9
Fertilization rate (%)	81	83	80
No. of embryos transferred	3.5 ± 1.1	2.9 ± 0.9	2.6 ± 0.8
Clinical pregnancy rate/transfer (%)	35	21	28
Delivery rate/transfer (%)[a]	27	12	20
Miscarriage rate (%)	22	44	28
Implantation rate (%)	14	9	13
Cycles with freezing (%)	30	18	27
Cryo clinical pregnancy rate/transfer (%)	24	20	17
Cryo implantation rate (%)	10	7	7

[a] $P<.01$.

Arslan. Ovarian stimulation and IVF. Fertil Steril 2005.

with either a GnRH agonist (luteal suppression) or a GnRH antagonist resulted in a high pregnancy rate and optimization of the total reproductive potential in intermediate responders. High responders also had a favorable and similar outcome using an OC/GnRH agonist overlap regimen or a GnRH agonist protocol in combination with recombinant FSH. On the other hand, poor responders achieved much lower success rates irrespective of the chosen regimen, which consisted of high-dose gonadotropins in combination with a GnRH micro-flare protocol, a GnRH antagonist, or a stop GnRH agonist protocol.

Fine-tuning of COH can be performed presently with the available battery of hormonal preparations and adjuvant therapies. In addition, new developments in the horizon may bring novel alternatives including more bioactive gonadotropin agonists with effects of variable duration (126). However, controversies still exist in the clinical setting. The debate continues on the addition of LH to cycles stimulated with a combination of GnRH agonist and recombinant FSH; a GnRH agonist suppression protocol, such as the one we have described, appears not to be needed for a successful outcome in intermediate and high responders. Conversely, an LH addition might be considered in low responders and in patients pretreated with OCs or following a more severe pituitary GnRH agonist suppression. Controlled studies are needed to confirm whether LH is needed in those clinical scenarios and which preparation achieves optimized results.

There is also controversy as to the use of flexible (individualized) or fixed regimens when using a GnRH antagonist. Other approaches such as early initiation of the GnRH antagonist (on day 1 of stimulation or in the preceding luteal phase) are also currently being evaluated (93, 127).

The ultimate goal in IVF is to achieve the transfer of a high quality embryo to the uterine cavity, thereby providing the infertile couple with their maximum chance of conception. The present incidence of multiple pregnancies, a true "ongoing epidemic" (116), underscores the need for improved, noninvasive methods to assess preimplantation embryo quality. Human embryo research has demonstrated that amino acid turnover has the potential to select viable embryos (128). It remains to be determined if measurements of metabolism and/or other secreted proteins by cultured human embryos can be used as biomarkers of developmental competence to identify the embryos with highest implantation potential.

It has been speculated that a possible cause for the rates of implantation observed in IVF cycles might be an impairment of endometrial receptivity due to high concentration of sex steroids resulting from COH (129). Histologic advancement of endometrial glandular/stromal compartments has been a common feature of IVF cycles stimulated with gonadotropins, with and without use of adjuvant therapy with GnRH agonists (130, 131). Furthermore, recent data have suggested that the use of GnRH antagonists in some COH regimens might result in compromised implantation rates (132). We recently demonstrated that, at the onset of the window of implantation and compared with natural cycles, COH resulted in histologic and pinopodes appearance advancement that was associated with small variations in endometrial gene expression as measured by micro-array technology. In addition, we identified differential gene expression when comparing recombinant FSH-stimulated cycles using a GnRH agonist against a GnRH antagonist (36). We are presently investigating whether some of these genes are relevant for implantation. More studies are needed to characterize endometrial gene/protein changes involved in the regulation of the timely establishment of the window of implantation and any potential effects of COH.

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