Does the degree of hypothalamic-pituitary-ovarian recovery after oral contraceptive pills affect outcomes of IVF/ICSI cycles receiving GnRH-antagonist adjuvant therapy in women over 35 years of age?

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Abstract

Purpose To evaluate if the degree of recovery of serum gonadotropins after oral contraceptive pills (OCP) pretreatment has an impact on ovarian response in GnRH-antagonist IVF cycles in women of advanced maternal age.

Methods In this retrospective cohort study, we included 98 women 35–42 years undergoing their first IVF cycle receiving gonadotropins and a fixed GnRH-antagonist adjuvant protocol. Data analysis was carried out according to changes in serum FSH, LH and estradiol (E_2) levels (basal and post-OCP) divided in quartiles, and also according to absolute levels. The main outcomes were peak serum E_2 , number of mature oocytes retrieved, length of stimulation, and amount of gonadotropins used.

Results By quartile analysis, patients with the highest levels of serum gonadotropins suppression and also patients with gonadotropin rebound needed larger amounts of LH during

the treatment. On the other hand, women with absolute suppression of FSH/LH had increased length of stimulation. **Conclusions** The results of this study provide data that assist in clinical management. Gonadotropin serum levels after OCP treatment provide information for optimization of supplementation with LH in GnRH-antagonist cycles in women over age 35.

Keywords GnRH antagonist · Hypothalamic-pituitary suppression · IVF · ICSI · Oral contraceptive pills

Introduction

Recently, it has been advocated that the use of GnRH-antagonist protocols in IVF/ICSI cycles has some advantages over GnRH-agonists, such as lowering the incidence of ovarian hyperstimulation syndrome and subsequent reduction in cycle's cancellation [2]. However the benefit of using oral contraceptive pills (OCP) prior to gonadotropins in a GnRH-antagonist protocol is still controversial. Pretreatment of IVF cycles with OCP has been suggested to result in attenuation of the FSH rise and induction of a more homogeneous follicular cohort, assisting synchronization of the follicular development with prolongation of the FSH window, and also preventing the occurrence of spontaneous LH-surges [7, 11, 27].

Despite these apparent benefits, a recent Cochrane review [29] showed that the use of OCP in patients receiving a GnRH-antagonist protocol was associated with fewer clinical pregnancies and higher amounts of gonadotropin doses needed, particularly in good prognosis patients, compared to no OCP use. On the other hand, in low responders GnRH-antagonist with OCP pretreatment appeared to be at least as

Capsule Variable changes in serum gonadotropin levels after OCP affect outcomes of GnRH-antagonist treated IVF/ICSI cycles

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effective as GnRH-agonists (long protocol) and superior in some aspects to GnRH-antagonists without OCP [14].

The activity of the hypothalamic-pituitary-ovarian axis in the pill-free interval during use of low-dose combined oral contraceptives in fertile young women has been characterized [31]. If and how the hypothalamic-pituitary recovery affects ovarian stimulation in infertile women undergoing IVF, particularly in low responders, needs further clarification.

In addition to potential biological effects that OCP pretreatment may offer in a GnRH-antagonist cycle, its use allows for more optimized scheduling which is convenient for the clinic and the patient. As always, convenience and benefits should be weighed against any deleterious effects of the intervention.

It is well established that there is a decreased chance of conception according to increasing woman's age, and that a more dramatic decline occurs after 35 years [6, 8]. It has been suggested that GnRH-antagonists may offer some advantages over other protocols for poor-responding women including those having low response associated with advanced maternal age [19]. On the other hand, a recent Cochrane review did not support the routine use of any particular regimen for this subgroup of patients [26]. In our center, most of the patients that are prospectively identified as having a compromised ovarian response and/or a poor prognosis (i.e., age over 35 and/or decreased ovarian reserve) are stimulated with higher dose gonadotropins with a GnRH-antagonist adjuvant protocol [25]. Therefore, in this study we collected data from patients who were older than 35 years undergoing IVF/ICSI with a GnRH-antagonist in a fixed regimen to investigate the endocrine effects of OCP pretreatment. The aim was to determine if the degree of recovery/suppression of serum gonadotropin levels after OCP pretreatment significantly affected the ovarian response.

Methods

In this retrospective cohort study, we examined computerized IVF data from 2008 to 2010. We included all consecutive patients with ages between 35 and 42 years when attempting their first IVF/ICSI cycle using an OCP/gonadotropins/GnRH-antagonist protocol ($n=98$), with the exception of couples undergoing preimplantation genetic diagnosis/screening. It has been the policy of our program to use OCP pretreatment in all patients allocated to a GnRH antagonist protocol in women ≥ 35 years. In addition, because of anticipated poor prognosis due to advanced maternal age, also associated with a low ovarian reserve in some cases defined as basal cycle day 3 FSH levels ≥ 10 mIU/ml, or estradiol (E_2) >90 pg/ml [3, 25, 28], or a high FSH:LH ratio (>3) [4], these patients were subjected to an initial high dose of gonadotropins. All included patients were of

advanced maternal age (35–42 years), and some had demonstrated low ovarian reserve, while others suffered from other etiologic diagnosis in addition to advanced age.

Patients were pretreated with oral ethinyl estradiol (E_2) $30\text{ }\mu\text{g}$ + desogestrel $150\text{ }\mu\text{g}$ for 21 days (range 18–23 days) during the cycle prior to scheduled IVF/ICSI, and ovarian stimulation was initiated 4 days after discontinuation of the pill. All patients were stimulated with a combination protocol consisting of recombinant FSH Gonal-F, Serono or Follistim, Schering-Plough) and human menopausal gonadotropin (Menopur, Ferring Pharmaceuticals), in a 2:1 or 3:1 ratio, given SC [22]. The starting FSH dose was individualized to 300 to 450 IU per day according to the attending's decision based on prospective identification of response based on age and ovarian reserve. The highest total gonadotropin dose was 600 IU/day, and the highest LH dose was 150 IU/day [20]. Gonadotropin doses were adjusted according to serum E_2 levels and follicle development measured with transvaginal ultrasound every 2–3 days. A daily dose of 0.25 mg of GnRH antagonist (Ganirelix, Schering Plough or Cetrotide, Serono) was administered SC from the morning of day 6 of stimulation onward [17]. Recombinant hCG $250\text{ }\mu\text{g}$ was administered SC when 2–3 leading follicles reached 17 mm in diameter. Transvaginal ultrasound-guided oocyte retrieval was performed 34–35 h later.

Standard IVF insemination or ICSI were performed in 19.4 % and 80.6 % of cycles, respectively. The highest quality of the transferred embryos was diagnosed by morphology score and cleavage rate based on the criteria of Veeck (scoring 1–5, 1= highest grade) [32]. Embryo transfer was performed on day 3 after retrieval. It is the policy of our program to transfer 2 embryos in a first attempt in all women under 43 years [3, 10]. If only 1–2 embryos were available, embryo transfer was performed on day 2. Progesterone supplementation (micronized progesterone 200 mg tid vaginally) was initiated the day after oocyte retrieval. Estrogen supplementation with 17β estradiol (1 mg per day vaginally) was started 5 days after retrieval. Serum hCG was evaluated 13 days after embryo transfer, and an ultrasound was performed at 7 weeks to confirm the presence of a gestational sac with a fetal heart beat, defined as a clinical pregnancy. Sex steroids supplementation was continued until week 8 of pregnancy. Clinical miscarriages were noted and all other pregnancies were considered ongoing (>28 weeks).

Serum FSH, LH and E_2 were collected on day 3 of a previous basal cycle as the baseline (within 3 months), and at the start of stimulation (4 days after the last OCP). Hormone levels were measured with a microparticle enzyme immunoassay (MEIA-IMX: Abbott Laboratories, Abbott Park, IL). The intra-assay coefficients of variation were 4.3, 4.1, and 6.1 % for FSH, LH, and E_2 , respectively. The inter-assay coefficients of variation were 4.9, 5.8, and 8.2 %

for FSH, LH, and E_2 , respectively. The lower limits of sensitivity were as follows: LH=1.0 mIU/mL, FSH=1.0 mIU/mL, and E_2 =25 pg/mL, respectively. The regression equations to convert RIA to IMX are as follows: IMX FSH=0.46×RIA−2.2; IMX LH=0.3×RIA−1.1; IMX E_2 =1.26×RIA−1.5.

Statistical analysis

Changes in serum FSH, LH and E_2 levels were analyzed in relation to primary outcomes (ovarian response parameters including peak serum E_2 , number of mature oocytes retrieved, length of stimulation, and amount of gonadotropins used) and to secondary outcomes (pregnancy results) after categorization into quartiles (Q1, Q2, Q3, and Q4). Outcomes were also analyzed according to absolute values of FSH and LH serum levels after OCP treatment. For this purpose patients were sub-divided into two groups, namely a suppressed (FSH and LH ≤1.2 mIU/mL) [5, 30] and a non-suppressed (FSH and LH >1.2 mIU/mL) group.

The statistical analysis was carried out by an independent statistician (BH) using SAS version 9.2. Bivariate associations involving categorical variables were analyzed using Pearson's Chi-square test, Fisher's exact test or the Cochran-Armitage trend test, where appropriate. Bivariate associations involving continuous variables were analyzed using Kruskal-Wallis test and post-hoc Wilcoxon's signed rank tests were performed for statistically significant associations. Possible effects of suppressed LH levels on follicular synchrony were analyzed by unpaired *t*-test, or contingency table, as appropriate. We considered $P<0.05$ as significant. Data were presented as percentages or means ± standard deviation.

Results

Patients' baseline characteristics and IVF/ICSI cycles major outcomes are shown in Table 1. Figure 1 illustrates how FSH serum levels varied after OCP treatment among the patients, with some individuals showing values similar to the basal levels (recovery), others showing higher levels or rebound, and finally others with lower than basal levels (suppression).

FSH serum level variations (after and before OCP) were statistically divided into quartiles as follows: Q1: <−4.9 mIU/mL ($n=19$), Q2: −4.9 to −2.2 mIU/mL ($n=28$), Q3: −2.3 to 1.1 mIU/mL ($n=24$) and Q4: ≥1.2 mIU/mL ($n=27$). Table 2 shows the effect of these variations on primary and secondary outcomes. All groups had similar mean age and FSH baseline serum levels. Overall, there were no significant differences regarding the peak E_2 , number of mature oocytes retrieved, length of stimulation or amount of recombinant FSH used among groups. Nevertheless, patients with highest degree of suppression (Q1) and patients with rebound (Q4)

Table 1 Patients' characteristics and IVF/ICSI cycle outcomes

Age (years)	37±1.4
Body Mass Index (kg/m ²)	27.6±7.3
	N (%)
< 25	43 (44)
25–29.9	33 (34)
≥ 30	22 (22)
Cause of infertility	N (%)
Tubal	13(13.3)
Endometriosis	7(7.1)
Ovarian	13(13.3)
Male	13 (13.3)
Unexplained	12 (12.2)
Uterine	13 (13.3)
Multifactorial	27 (27.5)
Day 3 basal serum levels	
FSH (mIU/ml)	6.4±1.6
LH (mIU/ml)	4.9±2.6
E_2 (pg/ml)	47.8±26.4
Post-OCP serum levels	
FSH (mIU/ml)	5.4±3.8
LH (mIU/ml)	3.2±2.6
E_2 (pg/ml)	33.6±16.2
Peak E_2 (pg/ml)	1895±1223
Number of mature oocytes retrieved	9.3±5.5
Length of stimulation (days)	9.5±1.1
Amount of FSH used (IU)	3721±1412
Amount of LH used (IU)	974±438
Fertilization method	N (%)
IVF	19 (19.4)
ICSI	79 (80.6)
Highest embryo grade	2.5±0.7
Cycle outcome	N (%)
Pregnant	23 (23.5)
Not pregnant	67 (68.4)
Miscarriages	8 (8.1)
Cycle cancellation	N (%)
Cancelled	4 (4)
Not cancelled	94 (96)

needed significantly more LH than those with moderate suppression (Q2) and recovery of baseline FSH serum levels (Q3).

There were no differences regarding highest quality of the transferred embryos, pregnancies, or cancellation rates between the groups. Analysis of the impact of LH and E_2 changes (also in quartiles) demonstrated similar patterns and did not show any other additional significant effects than those presented for FSH (data not shown).

For the second analysis of the data, patients with gonadotropin suppression analyzed as dichotomous variables (FSH

Fig. 1 Basal and post-OCP treatment serum FSH levels

and LH serum levels <1.2 mIU/mL) after OCP pretreatment showed significantly longer duration of gonadotropin stimulation (Table 3). In addition, we examined outcomes of possible follicular asynchrony between LH-suppressed and non-suppressed groups as determined by (i) proportion of mature oocytes/total number of recovered oocytes, (ii) total number fertilized (normal diploid fertilization)/total number of mature eggs inseminated or injected, and (iii) proportion of patients with top quality embryos (grades 1–2). No significant differences were observed for any of these parameters among the two groups.

Discussion

The current study showed that the evaluation of the degree of hypothalamic-pituitary-ovarian recovery of endogenous gonadotropin serum levels after OCP pretreatment in women of advanced maternal age (range 35–42) using a GnRH-antagonist may help in ovulation stimulation management.

To the best of our knowledge, this is the first study to examine the impact of the degree of hypothalamic-pituitary recovery (post-OCP compared to basal levels) on cycle outcomes in gonadotropin-stimulated IVF/ICSI patients using a fixed GnRH-antagonist regimen.

GnRH antagonist cycles have been largely used; however, it has been difficult to establish the optimal protocol because of the large variation of employed regimens used to study its efficacy [9, 13]. A recent Cochrane review [29] evaluated whether pre-treatment with combined OCPs, progestogens or estrogens affected outcomes in ART, and the results favored the use of progestogens. In addition to discussions about the pretreatment of GnRH antagonist cycles, the best day after the OCP withdrawal to start gonadotropins administration, which is usually between days 2 and 5, is also controversial [7, 16, 27]. It is possible that the duration of the pill-free period may affect cycle outcomes, depending on hormones recovery during these days [7, 31]. Hiurne et al. claimed that an earlier start is associated with stronger gonadotrophin suppression and less large follicles than later start; however, this was based on unpublished data [13].

In young fertile women undergoing OCP treatment, hypothalamic-pituitary recovery typically occurs by days 4–5 [7, 27, 31]. Cédric-Durnerin et al. showed that a 5-day free interval after OCP (ethinyl estradiol 30 μ g+desogestrel 150 μ g) is sufficient for gonadotropin recovery in young infertile patients [7]. Figure 1 illustrates how FSH levels varied markedly (recovery, rebound or suppression) after use of OCP in our study population. The reason for this remarkable variability, which was independent of the basal FSH and age, needs to be clarified, but it is speculated that dysregulation of ovarian factors could be the underlying mechanism, as these patients are of advanced maternal age. Nevertheless, it is now well known from animal and human studies that the

Table 2 Change in FSH levels (post- OCP compared to basal) in quartiles and impact on IVF/ICSI outcomes

	FSH (mIU/ml) change in quartiles				P
	Q1: <-4.9 ($n=19$)	Q2: $-4.9-<-2.3$ ($n=28$)	Q3: $-2.3-<1.2$ ($n=24$)	Q4: $1.2+$ ($n=27$)	
Age (years)	37.2 ± 1.2	37.3 ± 1.7	36.9 ± 1.5	37.1 ± 1.7	0.84 ^a
Baseline FSH	7.2 ± 1.1	6.4 ± 1.3	6.3 ± 1.7	5.8 ± 1.9	0.12 ^a
Peak E ₂ (pg/ml)	1436 ± 679	2277 ± 1487	1616 ± 762	2026 ± 1414	0.17 ^a
Number of mature oocytes retrieved	8.8 ± 5.1	10.6 ± 6.4	8.0 ± 4.7	9.2 ± 5.2	0.48 ^a
Length of stimulation (days)	9.8 ± 1.1	9.7 ± 1.1	9.2 ± 1.0	9.5 ± 1.2	0.15 ^a
Amount of FSH used (IU)	4070 ± 1329	3430 ± 1400	3646 ± 1169	3989 ± 1700	0.42 ^a
Amount of LH used (IU)	1195 ± 567	846 ± 335	804 ± 352	1150 ± 392	0.006 ^{a,c}
Highest grade embryo	2.56 ± 0.70	2.54 ± 0.76	2.43 ± 0.66	2.43 ± 0.59	0.87 ^a
Clinical pregnancies	22.2 %	26.9 %	30.4 %	26.1 %	0.31 ^b
Cycle cancellation	0.0 %	7.1 %	4.2 %	3.7 %	0.78 ^b

^a Kruskal-Wallis test. ^b Cochran-Armitage trend test. ^c Post-hoc analysis for amount of LH used by Wilcoxon's rank sum test: Q1 vs. Q2 ($P=0.14$); Q2 vs. Q3 ($P=0.36$); Q3 vs. Q4 ($P=0.0022$); Q1 vs. Q4 ($P=0.82$); Q1 vs. Q3 ($P=0.0098$); Q2 vs. Q4 ($P=0.031$)

Table 3 Effect of FSH and LH suppression after OCP treatment on IVF/ICSI outcomes

	Day 3 FSH (mIU/ml) and LH (mIU/ml) (dichotomous)		
	≤ 1.2 (n=17)	> 1.2 (n=60)	P
Age (years)	38.0±1.4	37.6±1.7	0.74 ^a
Basal FSH	5.7±1.4	6.5±1.7	0.45 ^a
Peak E ₂ (pg/ml)	1740±982	2023±1368	0.55 ^a
Number of mature oocytes retrieved	9.8±6.7	9.3±5.6	0.90 ^a
Length of stimulation (days)	10.2±1.1	9.4±1.1	0.028 ^a
Amount of FSH used (IU)	3877±1422	3834±1464	0.95 ^a
Amount of LH used (IU)	1119±597	986±395	0.46 ^a
% mature eggs/cohort	0.8±0.1	0.85±0.1	0.9 ^d
# fertilized eggs/# inseminated eggs	0.6±0.1	0.5±0.1	0.6 ^d
Highest grade embryo	2.2±0.7	2.5±0.6	0.09 ^a
Patients with top quality embryos	64 %	54 %	0.6 ^e
Clinical pregnancies	50 %	20 %	0.026 ^c
Cycle cancellation	5.9 %	3.3 %	0.53 ^c

^a Wilcoxon's rank sum test.^b Pearson's Chi-square test.^c Fisher's exact test. ^d T-test.^e Contingency table

transition towards menopause involves not only the loss of ovarian follicles but also a dysregulation of E₂ feedback mechanisms, and that this hypothalamic-pituitary dysfunction contributes to the onset and progression of reproductive senescence independent of ovarian failure [24]. Aberrant responsiveness of the hypothalamic pituitary axis to estrogen feedback and the subsequent generation of abnormal patterns of gonadotropin release (normal, attenuated, and failed LH surge induction under E₂ positive feedback conditions) may in itself accelerate ovarian follicular exhaustion [15]. Moreover, it is hypothesized that recovery from prolonged hypothalamic amenorrhea, which occurs after use of oral contraceptives, in part mimics hormonal sequelae of puberty characterized by temporary overshoot of FSH levels [23], which here could explain some of the cases that presented with FSH rebound.

In our study, patients that failed hypothalamic-pituitary recovery after OCP and that had the highest levels of suppression (Q1) had higher LH requirement. Interestingly, when gonadotropin suppression was analyzed as a dichotomous variable (cut-off of 1.2 mIU/mL), the highly suppressed patients demonstrated a higher pregnancy rate. This has to be interpreted cautiously as per the small sample size for the comparison, but the data strongly suggest that suppressed patients that are treated with high FSH and LH doses (in ratios of 2:1 or 3:1) can still achieve acceptable rates of pregnancy.

Patients with gonadotropin rebound after OCP (Q4) also required a higher LH dose, but that was the only noticeable change. The reason why these patients need more LH than patients with moderate gonadotropin suppression (Q2) and than patients with gonadotropin recovery (Q3) is unknown, but it may be important to achieve similar outcomes.

Taken together, the results of our study provide data that assist in clinical management. The comparison of serum gonadotropins on basal day 3 and post-OCP identifies the more

suppressed patients as well as the patients with gonadotropin rebound, who are the ones that will require higher doses of LH. Furthermore, these data indirectly provide support for the use of LH supplementation to patients with suspected poor response due to age over 35 (advanced maternal age). A recent Cochrane review showed that poor responders have higher pregnancy rates when they received recombinant LH, nevertheless that was demonstrated in GnRH-agonist cycles [22]. Moreover, it was observed that oocyte donors using GnRH antagonist protocol, LH supplementation improved the possibilities of gestation for the recipients [1].

There are a few studies relating cycle day 8 LH serum levels and pregnancy outcomes in GnRH antagonist cycles without OCP pretreatment. Kolibianakis et al., [18] showed that profound suppression of LH after GnRH antagonist administration (day 8 stimulation) is associated with a significantly higher chance of achieving an ongoing pregnancy. On the other hand, Meldrum et al. showed that patients with low serum LH levels on day of hCG administration had higher early pregnancy loss [21]. Furthermore, a recent meta-analysis [12] demonstrated no relation between LH serum levels and the likelihood of ongoing pregnancy in young patients. In our analysis of absolute FSH and LH serum levels after OCP treatment, we observed an increase in length of stimulation in suppressed patients, but we also saw an increase in pregnancy rates. Perhaps, if low LH levels have a real negative impact on cycle outcome in GnRH antagonist cycles, we overcame this problem supplementing it for all patients.

The main limitation of the current study is dependent upon its retrospective nature (i.e., no prospective allocation of groups to different LH doses). Nevertheless, it sheds light on the value of measurement of gonadotropins in the pill-free interval after OCP in order to guide management. It is concluded that gonadotropin serum levels after OCP treatment

may provide additional information for optimization of ovulation induction and supplementation with LH in GnRH-antagonist cycles in women over age 35.

References

1. Acevedo B, Sanchez M, Gomez JL, Cuadros J, Ricciarelli E, Hernandez ER. Luteinizing hormone supplementation increases pregnancy rates in gonadotropin-releasing hormone antagonist donor cycles. *Fertil Steril*. 2004;82:343–7.
2. Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, Abou-Setta AM. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev*. 2011;CD001750.
3. Arslan M, Bocca S, Mirkin S, Barroso G, Stadtmayer L, Oehninger S. Controlled ovarian hyperstimulation protocols for in vitro fertilization: two decades of experience after the birth of Elizabeth Carr. *Fertil Steril*. 2005;84:555–69.
4. Barroso G, Oehninger S, Monzo A, Kolm P, Gibbons WE, Muasher SJ. High FSH:LH ratio and low LH levels in basal cycle day 3: impact on follicular development and IVF outcome. *J Assist Reprod Genet*. 2001;18:499–505.
5. Björcke S, Fedorcsak P, Abyholm T, Storeng R, Ertzeid G, Oldereid N, Omland A, Tanbo T. IVF/ICSI outcome and serum LH concentration on day 1 of ovarian stimulation with recombinant FSH under pituitary suppression. *Hum Reprod*. 2005;20:2441–7.
6. Cai QF, Wan F, Huang R, Zhang HW. Factors predicting the cumulative outcome of IVF/ICSI treatment: a multivariable analysis of 2450 patients. *Hum Reprod*. 2011;26:2532–40.
7. Cedrin-Durnerin I, Bstandig B, Parneix I, Bied-Damon V, Avril C, Decanter C, Hugues JN. Effects of oral contraceptive, synthetic progestogen or natural estrogen pre-treatments on the hormonal profile and the antral follicle cohort before GnRH antagonist protocol. *Hum Reprod*. 2007;22:109–16.
8. Centers for Disease Control and Prevention. 2009 Assisted Reproductive Technology and Success Rates. National Summary and Fertility Clinic Reports 2007.
9. Devroey P, Aboulghar M, Garcia-Velasco J, Griesinger G, Humaidan P, Kolibianakis E, Ledger W, Tomas C, Fauser BC. Improving the patient's experience of IVF/ICSI: a proposal for an ovarian stimulation protocol with GnRH antagonist co-treatment. *Hum Reprod*. 2009;24:764–74.
10. Dowling-Lacey D, Jones E, Mayer J, Bocca S, Stadtmayer L, Oehninger S. Elective transfer of two embryos: reduction of multiple gestations while maintaining high pregnancy rates. *J Assist Reprod Genet*. 2007;24:11–5.
11. Gonen Y, Jacobson W, Casper RF. Gonadotropin suppression with oral contraceptives before in vitro fertilization. *Fertil Steril*. 1990;53:282–7.
12. Griesinger G, Shapiro DB, Kolibianakis EM, Witjes H, Mannaerts BM. No association between endogenous LH and pregnancy in a GnRH antagonist protocol: part II, recombinant FSH. *Reprod Biomed Online*. 2011;23:457–65.
13. Huirne JA, Homburg R, Lambalk CB. Are GnRH antagonists comparable to agonists for use in IVF? *Hum Reprod*. 2007;22:2805–13.
14. Kim CH, Jeon GH, Cheon YP, Jeon I, Kim SH, Chae HD, Kang BM. Comparison of GnRH antagonist protocol with or without oral contraceptive pill pretreatment and GnRH agonist low-dose long protocol in low responders undergoing IVF/intracytoplasmic sperm injection. *Fertil Steril*. 2009;92:1758–60.
15. Klein NA, Battaglia DE, Fujimoto VY, Davis GS, Bremner WJ, Soules MR. Reproductive aging: accelerated ovarian follicular development associated with a monotropic follicle-stimulating hormone rise in normal older women. *J Clin Endocrinol Metab*. 1996;81:1038–45.
16. Kolibianakis EM, Papanikolaou EG, Camus M, Tournaye H, Van Steirteghem AC, Devroey P. Effect of oral contraceptive pill pretreatment on ongoing pregnancy rates in patients stimulated with GnRH antagonists and recombinant FSH for IVF. A randomized controlled trial. *Hum Reprod*. 2006;21:352–7.
17. Kolibianakis EM, Venetis CA, Kalogeropoulou L, Papanikolaou E, Tarlatzis BC. Fixed versus flexible gonadotropin-releasing hormone antagonist administration in in vitro fertilization: a randomized controlled trial. *Fertil Steril*. 2011;95:558–62.
18. Kolibianakis EM, Zikopoulos K, Schiettecatte J, Smitz J, Tournaye H, Camus M, Van Steirteghem AC, Devroey P. Profound LH suppression after GnRH antagonist administration is associated with a significantly higher ongoing pregnancy rate in IVF. *Hum Reprod*. 2004;19:2490–6.
19. Mahutte NG, Arici A. Role of gonadotropin-releasing hormone antagonists in poor responders. *Fertil Steril*. 2007;87:241–9.
20. Meldrum DR, Schoolcraft W. Microdose flare versus antagonist for poor responders. *Fertil Steril*. 2010;93:e35. author reply e36.
21. Meldrum DR, Scott Jr RT, Levy MJ, Alper MM, Noyes N. Oral contraceptive pretreatment in women undergoing controlled ovarian stimulation in ganirelix acetate cycles may, for a subset of patients, be associated with low serum luteinizing hormone levels, reduced ovarian response to gonadotropins, and early pregnancy loss. *Fertil Steril*. 2009;91:1963–5.
22. Mochtar MH, Van der V, Ziech M, van Wely M. Recombinant Luteinizing Hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. *Cochrane Database Syst Rev*. 2007;CD005070.
23. Murphy MF, Campbell MJ, Bone M. Is there an increased risk of twinning after discontinuation of the oral contraceptive pill? *J Epidemiol Commun Health*. 1989;43:275–9.
24. Neal-Perry G, Nejat E, Dicken C. The neuroendocrine physiology of female reproductive aging: An update. *Maturitas*. 2010;67:34–8.
25. Oehninger S. Ovulation induction in IVF. *Minerva Ginecol*. 2011;63:137–56.
26. Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhattacharya S. Interventions for 'poor responders' to controlled ovarian hyperstimulation (COH) in in-vitro fertilisation (IVF). *Cochrane Database Syst Rev*. 2010;CD004379.
27. Rombauts L, Healy D, Norman RJ. A comparative randomized trial to assess the impact of oral contraceptive pretreatment on follicular growth and hormone profiles in GnRH antagonist-treated patients. *Hum Reprod*. 2006;21:95–103.
28. Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z. Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. *Fertil Steril*. 1989;51:651–4.
29. Smulders B, van Oirschot SM, Farquhar C, Rombauts L, Kremer JA. Oral contraceptive pill, progestogen or estrogen pre-treatment for ovarian stimulation protocols for women undergoing assisted reproductive techniques. *Cochrane Database Syst Rev*. 2010;CD006109.
30. The European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose-finding study. *J Clin Endocrinol Metab*. 1998;83(5):1507–14.
31. van Heusden AM, Fauser BC. Activity of the pituitary-ovarian axis in the pill-free interval during use of low-dose combined oral contraceptives. *Contraception*. 1999;59:237–43.
32. Veeck LL. Extracorporeal maturation: Norfolk, 1984. *Ann N Y Acad Sci*. 1985;442:357–67.