Sequential clomiphene citrate and human menopausal gonadotrophin with intrauterine insemination: the effect of patient age on clinical outcome*

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The purpose of this investigation was to examine the influence of female and male partner age on pregnancy rates with sequential clomiphene citrate (CC) and human menopausal gonadotrophin (HMG) ovulation induction with intrauterine insemination (IUI) therapy after previous CC and IUI treatment failure. A total of 208 patients previously unable to conceive with CC/IUI therapy underwent 416 treatment cycles of sequential CC/HMG with IUI at a university fertility centre between May, 1991 and August, 1995. Clinical pregnancy rates, live birth rates, and the effect of female and male partner chronological age were retrospectively examined. Treatment with sequential CC/HMG with IUI resulted in clinical pregnancy rates ranging from 5.9 to 23.1% despite previous CC/IUI treatment failure. Clinical pregnancy rates, live birth rates, and cumulative pregnancy rates declined significantly in female patients ≥35 years of age compared to those <35 years of age. A statistically significant decline in clinical pregnancy rates could not be demonstrated as a function of increasing male partner age. Pregnancy rates in patients undergoing ovulation induction with sequential CC/HMG with IUI decline significantly with increasing female partner age.

Key words: clomiphene citrate/human menopausal gonadotrophin/ovulation induction/patient age/pregnancy rates

Introduction

Ovulation induction together with intrauterine insemination (IUI) remains a mainstay of infertility therapy. Protocols for controlled ovarian stimulation (COH) in patients undergoing ovulation induction therapy have frequently utilized clomiphene citrate (CC) as first-line therapy (Agarwal et al., 1996). CC remains the treatment of first choice in the management of infertility in anovulatory women and has also been shown to be efficacious in normally ovulating women with inadequate follicular or luteal development (Dickey and Holtkamp, 1996; Kousta et al., 1997). Furthermore, an improvement in pregnancy rates with CC has also been demonstrated when female partner weight and CC dose are appropriately adjusted (Dickey et al., 1997). Patients who fail to conceive with CC therapy have subsequently been treated with human menopausal gonadotrophins (HMG) (Dodson and Haney, 1991). Recently, sequential clomiphene citrate and human menopausal gonadotrophin (CC/HMG) therapy has become an increasingly utilized method of ovarian stimulation for patients who fail CC treatment (Kemmann and Jones, 1983; Rose, 1992; Dickey et al., 1993; Lu et al., 1996).

A significant reduction in the reproductive capacity of the human female occurs during the fourth decade of life (Gindoff and Jewelewicz, 1986; Menken et al., 1986; Pearlstone et al., 1992). Studies utilizing therapeutic donor insemination (Schwartz and Mayaux, 1982; Stovall et al., 1991), ovarian stimulation with HMG (Dodson and Haney, 1991; Brzechffa and Buyalos, 1997) and assisted reproductive technologies (Assisted reproductive technology in the United States and Canada, 1998) demonstrate a profound decline in pregnancy rates per treatment cycle in women ≥35 years of age. Although there is substantial evidence of the decline in reproductive capacity in women, there is limited information regarding the influence of male partner age in ovulation induction therapy. The objectives of this investigation were to determine the influence of female and partner age on clinical pregnancy and live birth rates in patients undergoing ovulation induction with sequential CC/HMG with IUI therapy after previous CC treatment failure.

Materials and methods

Subjects

The study population consisted of women aged 24–46 years and their male partners aged 26–56 years undergoing ovulation induction with sequential CC/HMG in combination with IUI at the Fertility Center of the University of California, Los Angeles between May 1, 1991 and August 1, 1995. Informed written consent was obtained before initiating therapy. A total of 416 consecutively completed treatment cycles in 208 patients were retrospectively evaluated. Prior to qualifying for inclusion in this study, patients met strict criteria for diagnosis of infertility with at least 1 year of unprotected intercourse and having previously undergone a minimum of three ovulatory treatment cycles with CC/IUI therapy without achieving conception. A midluteal serum progesterone >10 ng/ml was considered to be indicative of an ovulatory treatment cycle.

The infertility evaluation consisted of an assessment of ovulation,
semen analysis, evaluation of the luteal phase and confirmation of tubal patency (hysterosalpingogram and/or laparoscopy). Laparoscopy was performed in a selective manner consistent with published protocols (Navot et al., 1987; Collins and Rowe, 1989). Patients were classified into one of five diagnostic categories based on their principal diagnosis: i.e. ovulatory dysfunction, endometriosis, tubal, uterine and cervical factor, therapeutic donor insemination and unexplained infertility. Ovulatory dysfunction included patients with oligo-ovulation or anovulation secondary to either hyperprolactinaemia, hypothalamic amenorrhoea or polycystic ovary syndrome as diagnosed by standard criteria. Endometriosis was diagnosed at laparoscopy and classified according to the revised American Society for Reproductive Medicine classification system (The American Fertility Society, 1985).

All females, including those with tubal factor, had at least one patent Fallopian tube. Couples were classified as having unexplained infertility if there was no evidence of abnormality during their evaluation. Patients were diagnosed as having male factor subfertility by the results of at least two semen analyses separated by at least 3 months that confirmed any of the following abnormalities: sperm concentration <20×10^6/ml, motility <50% or normal morphology <50%. To avoid the confounding effect of poor semen quality all couples diagnosed with male factor either received insemination with donor sperm or were excluded from this analysis.

**Ovarian induction protocols**

Ovarian stimulation protocols utilized in this study employed sequential CC/HMG. In order to exclude residual cysts, a baseline transvaginal ultrasound was performed on cycle day 3 prior to initiating each treatment cycle. CC was administered in doses ranging from 50 to 100 mg/day for 5 consecutive days, with therapy initiated on cycle day 3 of either a spontaneous cycle or following progestin-induced withdrawal bleeding. On the day following the fifth day of CC administration, HMG therapy was initiated for 3 days at an individualized dose ranging from one to two ampoules. Each ampoule contained 75 IU of FSH and 75 IU of LH (Pergonal; Serono Laboratories, Randolph, MA, USA). A repeat transvaginal ultrasound was performed on cycle day 3 prior to initiating HMG therapy to confirm follicular development. Initial serum oestradiol concentration were subsequently obtained after 5 days of CC and 3 days of HMG therapy to confirm follicular development. For patients where the transvaginal ultrasound did not confirm a follicle(s) with a mean diameter of 18 mm, treatment with HMG was initiated for 3 days at an individualized dose ranging from one to two ampoules. Each ampoule contained 2000 IU of FSH and 2000 IU of LH (Fertinex; Miles Laboratories, Indianapolis, IN, USA). A repeat transvaginal ultrasound was performed 5 weeks following IUI in those women without menses to confirm pregnancy.

Inseminations were timed by the administration of human chorionic gonadotrophin (HCG) (5000 IU) (Profasi; Serono) when the lead follicle(s) attained a mean diameter of 18 mm. Single inseminations were performed ~36 h following HCG administration. A total volume of 0.3–0.5 ml was inseminated in all cases following a standard swim-up procedure as previously described (Dodson et al., 1987). All donor insemination cycles used cryopreserved semen. An additional 5000 IU of HCG was administered 5 days after IUI for luteal phase support. Initial serum HCG quantitations were performed 16 days following IUI in those women without menses to confirm pregnancy.

**Outcomes**

A clinical pregnancy was defined as the detection of fetal cardiac activity by transvaginal ultrasound scan performed 5 weeks following insemination in the presence of a rising HCG titre. Live births referred to pregnancies ending in the delivery of a viable infant. The clinical pregnancy rate was defined as the number of clinical pregnancies divided by the number of treatment cycles performed. The live birth rate was defined as the number of live births divided by the number of treatment cycles performed.

**Statistical methods**

Proportions were compared via χ² and exact permutational P values were computed. Logistic regression procedures were used to assess trends in proportions. A Kaplan–Meier life table analysis was used to estimate the cumulative pregnancy rates. Analysis of variance was used for comparison of means. Power calculations were performed using power and sample size analysis. Statistical significance was considered as P < 0.05.

**Results**

In this analysis, 416 consecutively completed ovulation induction cycles using sequential CC/HMG with IUI in 208 patients resulted in 59 clinical pregnancies and 52 live births for a clinical pregnancy rate of 14.2% and a live birth rate of 12.5% per treatment cycle. Of the 59 clinical pregnancies four were twin gestations and of the 52 live births four were twin gestations. There were no higher order multiple gestations. A mean of 2.0 treatment cycles per patient was performed (range: one to five treatment cycles). There was one case of severe ovarian hyperstimulation syndrome giving an overall incidence of 0.2% per treatment cycle.

**The influence of female partner age**

The significant decline observed in clinical pregnancy rates and live birth rates per treatment cycle with advancing female partner age is illustrated in Table I. The clinical pregnancy rate (23.1%) and live birth rate (21.8%) were significantly higher in the <35 year age group, compared to the 35–40 year age group (10.3%, 8.6%; P = 0.008) and the >40 year age group (5.9%, 3.5%; P < 0.001) respectively. No difference was observed when comparing the clinical pregnancy and live birth rates between the 35–40 and >40 year age groups. Furthermore, there were significant differences in both the clinical pregnancy rate (23.1 versus 8.8%; P < 0.001) and the live birth rate (21.8 versus 6.9%; P = 0.004) when the ages...
Figure 1. Cumulative pregnancy rates using Kaplan–Meier life table analysis for 208 infertile female patients undergoing sequential clomiphene citrate/human menopausal gonadotrophins with intrauterine insemination therapy stratified by the age of the female partner into <35, 35–40 and >40 year age groups (A) and into <35 and ≥35 year age groups (B).

The cumulative pregnancy rates of the study population stratified by the chronological age of the female partner at the time of treatment are depicted in Figure 1. Logistic regression analysis revealed a significant decline in the cumulative pregnancy rates after three treatment cycles with increasing chronological age of the female partner; 59% for women <35 years of age, 26% for women 35–40 years of age, and 21% for women >40 years of age (P < 0.004) (Figure 1A). Additionally, when the study population was stratified into women <35 and ≥35 years of age, a substantial decline in the cumulative pregnancy rate was observed (59% versus 25%; P = 0.001) (Figure 1B).

There was no significant difference observed between clinical pregnancy rates and infertility diagnoses as shown in Table II. A similar analysis of live birth rates versus infertility diagnoses demonstrated no significant differences. In addition, there was no statistically significant difference in female partner age between each of the five different infertility diagnoses. Treatment cycle parameters are shown in Table III stratified into groups by chronological age of the female partner and pregnancy outcome. No differences were observed in maximum oestradiol level, number of mature follicles, endometrial lining thickness, male partner age or inseminating sperm concentration between the non-pregnant and pregnant women within each age group (P ≥ 0.25). Additionally, no differences were observed in clinical pregnancy rates with different infertility diagnoses.

Table II. Infertility diagnosis and clinical pregnancy rate (CPR) per treatment cycle

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Non-pregnant</th>
<th>Pregnant</th>
<th>CPR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovulatory</td>
<td>69</td>
<td>16</td>
<td>18.8</td>
</tr>
<tr>
<td>Tubal, uterine and cervical</td>
<td>96</td>
<td>15</td>
<td>13.5</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>52</td>
<td>9</td>
<td>14.8</td>
</tr>
<tr>
<td>Donor insemination</td>
<td>13</td>
<td>3</td>
<td>18.8</td>
</tr>
<tr>
<td>Unexplained</td>
<td>127</td>
<td>16</td>
<td>11.2</td>
</tr>
</tbody>
</table>

There was no significant difference (P > 0.05) in clinical pregnancy rates with different infertility diagnoses.

The influence of male partner age

There was no significant difference in the mean inseminating sperm concentrations between different male partner age groups. The clinical pregnancy rates per treatment cycle as a function of male partner age, stratified into <30, 30–39, 40–49 and ≥50 year age groups, and controlling for female partner age, are shown in Table IV. Although a trend was observed (P = 0.06), a statistically significant decline in clinical pregnancy rates could not be demonstrated as a function of increasing male partner age. This may reflect insufficient statistical power due to sample size. The power to detect a difference of 10% in clinical pregnancy rates with increasing male partner age ranged from 0.08 to 0.09, a 25% difference from 0.20 to 0.24 and a 50% difference from 0.58 to 0.73 when controlling for female partner age.

Discussion

The goal of this investigation was to determine the influence of female and male partner age on pregnancy rates with an ovulation induction protocol utilizing sequential CC/HMG with IUI. This study illustrates that clinical pregnancy and live birth rates decline significantly when the female partner is >35 years of age. This report did not demonstrate a statistically significant difference in clinical pregnancy and live birth rates with increasing male partner age after controlling for the age of the female partner. Previously, we reported a decline in pregnancy rates with HMG/IUI therapy when the male partner was >40 years of age and the female partner was >35 years of age (Brzechffa and Buyalos, 1997). The inability to demonstrate a statistically significant difference in pregnancy rates as a function of male chronological age may reflect insufficient sample size. This study also suggests that treatment
protocols for CC/HMG therapy in these reports vary widely. HMG and HMG protocols (Ransom et al., 1993; Lu et al., 1997). The current study demonstrates that treatment with CC/IUI therapy ranging from 6.9 to 23% depending upon the age of the female partner (Brzechffa and Buyalos, 1997).

Ovulation induction with CC in combination with IUI is an established first-line therapy for the treatment of both ovulatory and anovulatory infertility diagnoses. The majority (80%) of conceptions with CC/IUI therapy occur in the first three treatment cycles (Agarwal and Buyalos, 1996). Additionally, we reported that treatment with CC/IUI therapy resulted in pregnancy rates ranging from 5 to 19% per treatment cycle contingent upon the age of the female partner (Agarwal and Buyalos, 1996). Recently, we documented pregnancy rates with HMG/IUI therapy ranging from 6.9 to 23% depending upon the age of the female partner (Brzechffa and Buyalos, 1997). The current study demonstrates that treatment with sequential CC/HMG results in clinical pregnancy rates ranging from 5.9 to 23.1%, despite prior CC treatment failure.

Patients who fail to conceive with CC therapy are often subsequently treated with HMG/IUI therapy. Similar pregnancy rates in patients undergoing ovulation induction utilizing sequential CC/HMG versus HMG ovulation induction have been reported (Kistner, 1966; Jarrell et al., 1981; Dickey et al., 1993; Lu et al., 1996), while one study has failed to demonstrate a similar pregnancy rate between sequential CC/HMG and HMG protocols (Ransom et al., 1996). However, protocols for CC/HMG therapy in these reports vary widely and do not consistently control for age of the female partner. Additional advantages of ovulation induction with sequential CC/HMG compared to HMG therapy alone include decreased HMG requirements, resulting in reduced cost and a decreased incidence of ovarian hyperstimulation syndrome and multiple gestations (Dickey et al., 1993; Lu et al., 1996; Ransom et al., 1996).

There are limited data addressing the influence of male partner age on pregnancy rates in patients undergoing ovulation induction therapy. One report which examined the role of sperm preparation and IUI reported a lower mean age in males (32 versus 35 years) whose female partners conceived (Galle et al., 1990). However, this study did not control for the age of the female partner and only 57 of 227 treatment cycles employed ovarian stimulation protocols. An epidemiological study which reviewed fecundity rates in fertile couples prior to 1911 in Ireland, which controlled for female partner age, reported decreased fecundity rates when the male was >42 years of age (Anderson, 1975). Furthermore, a study which reviewed the impact of male factors on fecundity rates in infertile couples demonstrated a decline in pregnancy rates for men >30 years of age (compared to males <30 years of age) in couples in which the female partner was >30 years of age (Ducot et al., 1988). Recently, we reported a marked decline in clinical pregnancy rates with HMG/IUI therapy with increasing male partner age (>40 years) after controlling for female partner age (Brzechffa and Buyalos, 1997). In addition, a study reported that the age of the male partner was the most significant prognostic factor for pregnancy in infertile couples undergoing ovarian stimulation with IUI therapy where the female partner was ≤43 years of age (Mathieu et al., 1995).

Alternatively, a study which examined couples undergoing in-vitro fertilization showed no relationship between the age of the male partner and pregnancy rates after controlling for female age (Piette et al., 1990). Thus, the significance of male partner age on pregnancy rates in infertile couples treated with different therapeutic regimens needs to be further clarified and is the source of additional study.

Although this study is limited by its retrospective design and lack of randomization to alternative treatment regimens, these data demonstrate that clinical pregnancy rates and live birth rates in couples undergoing ovulation induction with sequential CC/HMG profoundly decline with increasing female age.

### Table III. Cycle characteristics by age of the female partner

<table>
<thead>
<tr>
<th>Maximum oestradiol concentration (pg/ml)</th>
<th>&lt;35 years</th>
<th>≥35 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>975 ± 76</td>
<td>1064 ± 136</td>
</tr>
<tr>
<td>Pregnant</td>
<td>971 ± 43</td>
<td>849 ± 87</td>
</tr>
</tbody>
</table>

| Mature follicle number                | 2.8 ± 0.2 | 3.3 ± 0.2 |
| Endometrial lining (mm)              | 10.0 ± 0.3 | 10.4 ± 0.5 |
| Male partner age (years)             | 35.2 ± 0.6 | 34.3 ± 1.1 |
| Inseminating sperm concentration (>×10⁶/ml) | 35.3 ± 3.8 | 28.1 ± 3.7 |

### Table IV. Clinical pregnancy rates per treatment cycle grouped by female partner age and male partner age

<table>
<thead>
<tr>
<th>Female partner age (years)</th>
<th>Male partner age (years)</th>
<th>&lt;30</th>
<th>30–39</th>
<th>40–49</th>
<th>&gt;50</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>32.1</td>
<td>21.9</td>
<td>22.2</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>35–40</td>
<td>17.4</td>
<td>11.4</td>
<td>11.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>&gt;40</td>
<td>14.3</td>
<td>6.6</td>
<td>5.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Values represent clinical pregnancy rate (%).

Values are mean ± SEM.

No significant difference between values for non-pregnant and pregnant patients within each age group.
age, particularly $\geq 35$ years, and are not adversely affected by increasing male partner age.

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References


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