BACKGROUND: This study was undertaken to assess whether the use of clomiphene citrate in conjunction with albumin-separated sperm would alter the sex ratio (expressed as the proportion of males) towards females and, if so, whether this skewing was due solely to the induction of ovulation. METHODS: The sex ratios of 184 single and 42 twin births at five assisted reproduction biology clinics were determined. The normal approximation to the binomial distribution was used to determine significant differences between these sex ratios and the established sex ratios for single, twin and combined (single and twin) non- and ovulation-induced births. RESULTS: The non-ovulation-induced sex ratios for singletons (51.4%) and twins (50.2%) were greater than the treatment singleton (27.7%; \( P < 0.001 \)) and twin (33.3%; \( P < 0.01 \)) sex ratios respectively. Correspondingly, the non-induced sex ratio for combined births (51.4%) was greater than the treatment sex ratio (28.8%; \( P < 0.001 \)). The previously established induced singleton and twin sex ratios (48.1%) were lower than the non-induced sex ratio (51.4%), but higher than the treatment singleton (27.7%; \( P < 0.001 \)) or twin (33.3%; \( P < 0.03 \)) sex ratios. The ovulation-induced combined ratio (48.1%) was less than the non-induced combined (51.4%) sex ratio, although greater than the treatment combined sex ratio (28.8%; \( P < 0.001 \)). CONCLUSION: Clomiphene citrate in conjunction with albumin-separated sperm decreased the sex ratio; a reduction that was not exclusively due to induction of ovulation.

Key words: albumin separation/clomiphene citrate/sex ratio/sex selection/sperm

Introduction

The secondary sex ratio, i.e. the proportion of males to females at the time of birth, can be expressed as the percentage of males and is conventionally thought to be similar (51.4%) for both singleton births (Maconochie and Roman, 1997) and for the general population, which includes multiple births (Ericsson and Ericsson, 1999). The twin birth sex ratio (50.2%) is slightly less than the singleton and general population sex ratios (National Center for Health Statistics, 1995). Many factors have been shown to slightly alter sex ratios. These include ethnicity, season of the year, birth order, maternal and paternal age, specific diseases and nutrition (Ericsson and Ericsson, 1999). An additional variable which has been identified as skewing the sex ratio is gonadotrophin- and/or clomiphene-induced ovulations resulting in either single or twin births (48.1%) (James, 1985).

Treatment with clomiphene citrate (CC) prior to artificial insemination with albumin-separated sperm is currently used clinically for couples desiring a female child. This procedure involves isolation of progressively motile sperm that are morphologically normal using an albumin column (Ericsson and Ericsson, 1999) with resulting pregnancy and spontaneous abortion rates similar to those observed in the general population (Beernink et al., 1993). These selected sperm are artificially inseminated into a woman who has been treated with the ovulatory drug CC.

The purpose of this study was to determine whether the use of CC in combination with albumin-separated sperm would reduce the sex ratio and, if so, whether this decline was due entirely to induction of ovulation.

Materials and methods

Experimental design

The sex ratios of offspring of couples treated with CC prior to artificial insemination with albumin-separated sperm were compared with established sex ratios. Differences from the treatment sex ratios for the general population, single and twin births were established through comparison with the corresponding control sex ratios for combined (single and twin; 51.4%) (Ericsson and Ericsson, 1999), single (51.4%) (Maconochie and Roman, 1997) and twin (50.2%) (National Center for Health Statistics, 1995) births respectively. In
addition, the treatment singleton and twin sex ratios were compared with the sex ratio (48.1%) identified for gonadotrophin- and/or CC-induced ovulations resulting in either single or twin births (both 48.1%) (James, 1985).

**Subjects**

Couples who participated in this study were self-selected from five assisted reproduction biology clinics and were provided with information about the risks involved prior to using CC and albumin column-separated sperm. Clinics did not terminate pregnancies due to the presence of a male fetus. Female patients received 50 mg of CC on cycle days 5–9. A total of 184 single and 42 twin births resulted from the use of this procedure. The singleton \( (n = 10; \text{sex ratio } 30.0\%) \) and twin \( (n = 4; 0.0\% \text{sex ratio}) \) subject groups included births from couples seeking female sex selection due to their carrying a sex-linked disorder.

**Sperm preparation techniques**

Each semen sample was diluted with an equal amount of Tyrode’s salt solution (Sigma Chemical Co., St Louis, MO) and centrifuged at 300 g for 10 min. The washed sperm were resuspended in Tyrode’s salt solution at a concentration of \( 30 \times 10^6 \) sperm per ml. Aliquots of 0.5 ml portions, containing \( 15 \times 10^6 \) washed sperm with at least 40% motility, were then layered over a 1 ml solution of 7.5% human serum albumin (HSA) over 0.5 ml of 17.5% HSA (Alpha Therapeutic Corp., Los Angeles, CA, USA) for 1 h in an 8 × 75 mm glass column at room temperature. The 0.5 ml layer of sperm was removed and the columns allowed to stand for another 30 min. The remaining 7.5% HSA layer was then removed and discarded. The 17.5% layer was pooled, diluted with an equal volume of Tyrode’s salt solution and centrifuged at 300 g for 10 min. The sperm pellet was resuspended in 0.25 ml of Tyrode’s salt solution in preparation for artificial insemination (17% motile sperm recovery). The mean number of motile sperm (95%) used for insemination was \( 38 \times 10^6 \) (Beernink et al., 1993).

Intracervical or intrauterine insemination was utilized just prior to a rise in basal body temperature on the day following a LH surge in CC-treated patients.

**Statistical analysis**

Significant differences between treatment and established sex ratios were assessed using the normal approximation to the binomial distribution (Zar, 1999).

**Results**

The variation among centres in the single birth and twin sex ratios of children born to couples who used CC and albumin column-separated sperm for female sex selection is illustrated in Table I. The significant differences between established control sex ratios (with and without CC) and the treatment sex ratios are shown in Table II. The control (non-CC) sex ratios for singletons (51.4%) (Maconochie and Roman, 1997) and twins (50.2%) (National Center for Health Statistics, 1995) were greater than the treatment singleton (27.7%; \( P < 0.001 \)) and twin sex ratios (33.3%; \( P < 0.01 \)) respectively. Similarly, the control (non-CC) sex ratios for combined (single and twin; 51.4%) births (Ericsson and Ericsson, 1999) were greater than the treatment sex ratio (28.8%; \( P < 0.001 \)). The CC control sex ratios for singleton and twin (48.1%) births (James, 1985) were higher than the corresponding treatment singleton (27.7%; \( P < 0.001 \)) and twin (33.3%; \( P < 0.03 \)) sex ratios. This same trend was again observed when the CC control for combined (48.1%) births (James, 1985) was compared with the treatment combined sex ratio (28.8%; \( P < 0.001 \)).

**Discussion**

Some clinicians are doubtful as to the efficacy of using CC prior to artificial insemination with albumin-separated sperm (Ericsson and Ericsson, 1999) as a method of skewing the sex ratio towards females. In addition, other practitioners question whether the alteration of the sex ratio is due solely to the use of CC and not albumin-separated sperm (Martin, 1994). The reduction in the established sex ratio for the general population (single and twin) and single births from 51.4 (Ericsson and Ericsson, 1999) to 28.8 and 27.7% respectively, illustrates that this methodology is effective in increasing the number of females born. A similar reduction in the twin sex ratio from 50.2 (National Center for Health Statistics, 1995) to 33.3% shows that this sex selection procedure is effective for improving the chance of twin female births. The skewing of the sex ratio in favour of

**Table I.** Single birth and twin sex ratios reported by individual centres for children born to couples who used clomiphene citrate ovulation induction and albumin column-separated sperm for female sex selection

<table>
<thead>
<tr>
<th>Centre</th>
<th>Single births</th>
<th>Twin births</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.2 (69)</td>
<td>40.9 (22)</td>
</tr>
<tr>
<td>2</td>
<td>14.3 (14)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>36.1 (36)</td>
<td>16.7 (6)</td>
</tr>
<tr>
<td>4</td>
<td>38.0 (50)</td>
<td>28.6 (14)</td>
</tr>
<tr>
<td>5</td>
<td>20.0 (15)</td>
<td></td>
</tr>
</tbody>
</table>

**Table II.** The sex ratio of children born to couples who used clomiphene citrate (CC) and albumin column-separated sperm for female sex selection

<table>
<thead>
<tr>
<th>Births (n)</th>
<th>Treatment sex ratio (%)</th>
<th>Significance of difference from treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control sex ratio (%)</td>
<td>Control sex ratio (%)</td>
</tr>
<tr>
<td></td>
<td>without CC</td>
<td>with CC</td>
</tr>
<tr>
<td>Single (184)</td>
<td>27.7</td>
<td>51.4; ( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Twin (42)</td>
<td>33.3</td>
<td>50.2; ( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Combined (single and twin) (226)</td>
<td>28.8</td>
<td>51.4; ( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>
females by all centres illustrates that this procedure can be used successfully by different clinicians for female sex preselection.

The ovulatory agent, CC, does reduce the sex ratio as shown by the singleton, twin and combined (single and twin) CC-induced births (48.1%) (James, 1985) being lower than those identified for similar non-induced births (51.4%) (Maconochie and Roman, 1997; Ericsson and Ericsson, 1999). The further decrease in the sex ratio for single (27.7%), twin (33.3%) and combined (28.8%) births attributable to the use of CC and albumin-separated sperm reveals that sperm treatment is an essential component of this procedure for reducing the sex ratio.

We conclude that the use of CC in conjunction with albumin-separated sperm will alter the sex ratio towards females and that this skewing is not exclusively due to induction of ovulation.

References


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