Intrauterine insemination: effect of the temporal relationship between the luteinizing hormone surge, human chorionic gonadotrophin administration and insemination on pregnancy rates

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The optimal time period for intrauterine insemination (IUI) in relation to either luteinizing hormone (LH) surge or human chorionic gonadotrophin (HCG) administration leading to the best pregnancy rates has not been determined. In this study, 856 consecutive human menopausal gonadotrophin (HMG)-stimulated and 49 natural unstimulated IUI cycles carried out at a reproductive medicine unit affiliated with a tertiary centre were analysed in a retrospective fashion. There were three scenarios in the temporal relationship of the LH surge, HCG administration and artificial insemination. These were (group A) subjects who had an endogenous LH surge but were not given HCG; (group B) subjects who were given HCG after an observed LH surge, and (group C) subjects who were given HCG before the LH surge. The overall pregnancy rate (PR) was 16% per cycle. The PR was 9% in group A, 20% in group B and 14% in group C. The PR in group B was significantly better than group C ($P = 0.04$). In group B, the longer the time interval between the LH surge and HCG administration, the better the PR up to 20 h ($P = 0.025$); the timing of IUI based on the LH surge was not critical to the achievement of pregnancy within 3 days. In group C, PR improved with the increasing interval between HCG and IUI from <28 h up to 60 h. We conclude that a better PR is achieved if a spontaneous LH surge occurs before HCG administration, especially where the administration of HCG is delayed 8–20 h after an observed LH surge; the timing of IUI based on the LH surge is not critical to the achievement of pregnancy within 3 days.

Key words: HCG/HMG/infertility/intrauterine insemination/LH surge

Introduction
Therapeutic intrauterine insemination (IUI) is widely used for the empiric treatment of infertility. Stimulated IUI has been suggested by some authors to give significantly better results compared with timed intercourse (Hurst et al., 1992; Nan et al., 1994). The main hypothesis postulated to explain the success of this treatment has been related to the timing of the presence of an increased concentration of motile spermatozoa in the proximity of the oocyte (Moghissi, 1986). Furthermore, a higher pregnancy rate (PR) is achieved when IUI is combined with the use of human menopausal gonadotrophin (HMG) to stimulate multiple follicle development (Martinez et al., 1991; Irianni et al., 1993). The efficiency of IUI with HMG injection may be more evident in the treatment of mild or even moderate male factor, cervical factor, endometriosis or unexplained infertility (Chaffkin et al., 1991; Hurst et al., 1992; Nulsen et al., 1993; Gregoriou et al., 1995), but the contributing factors to maximize IUI PR remain unresolved. In this study we examine the influence of some factors on PR.

The timing of insemination in IUI programmes in relation to other major events around ovulation or most probably ovulation itself has been suggested as the most important variable affecting the success of this treatment (Allen et al., 1985). To maximize the chance of success, the timing of insemination needs to be closely related to the time of ovulation (Allen et al., 1985; Moghissi, 1986; Kenmamm et al., 1987). There have been some discrepancies in the literature regarding the time of ovulation after the onset of the luteinising hormone (LH) surge. In a multicentred collaborative study from the World Health Organization, it was found that ovulation occurred between 24 and 56 h after the onset of the LH surge (WHO, 1980). Another study found that ovulation occurred at a mean time of 27.3 h from onset of the LH surge (Garcia et al., 1981). Ovum retrieval 36–38 h from the initiation of the LH surge achieves good fertilization rates (Testart et al., 1981). Martinez et al. (1991a,b) suggested that a beneficial effect would arise if the final process of natural follicular maturation was allowed to occur following the spontaneous rise of LH. However, human chorionic gonadotrophin (HCG) is widely used in most IUI programmes to promote the final maturation of pre-ovulatory follicles and to time ovulation. Follicular rupture usually occurs ~36–48 h after HCG injection (Testart, 1990).

We aimed to test the hypothesis that a spontaneous LH surge gives a higher PR and that there was an optimal time period in relation to either LH surge or HCG injection during which insemination will achieve the best PR.

Materials and methods
Subject selection
This study was based on data collected from subjects treated in the IUI programme in the Reproductive Medicine Unit at the Queen Elizabeth Hospital during 1990–1995. During this period, 463 subjects underwent 856 completed cycles with HMG and HCG to induce ovulation (94%) and 49 natural unstimulated cycles (6%). Usually no more than three cycles were performed per subject.

In our Unit, IUI with low dose gonadotrophin stimulation was
mainly indicated for patients with unexplained infertility or moderate semen defect where at least two of the following criteria were generally met in two semen analyses: >15 × 10^6 spermatozoa/ml, 30–50% progressive motility and >15% normal morphology. All couples had >1 year of primary or secondary infertility and they were investigated by means of cycle tracking, which included a sperm cervical mucus contact test, tubal assessment (either by laparoscopy and dye or hysterosalpingogram) and sperm immobilization test for serum antisperm antibodies. Criteria for inclusion were: (i) female partner should be <45 years of age at the time of treatment and have a normal ovulatory history or ovulate in response to medication; (ii) female partner must have bilateral patent Fallopian tubes, demonstrated within the last 2 years; (iii) male partner must have had at least three semen analyses and one trial sperm washing within the last 2 years. In this study, we located 1090 treatment cycles initiated, including 185 cancelled cycles, not included in this study, and 905 cycles with insemination completed. Of the 905 cycles, 10 cycles (1%) were excluded for missing data and 15 cycles with missing the time of HCG administration were included in the overall analysis, but excluded from some calculations.

**Sperm preparation**

Patients were requested not to have intercourse for 3 days before the day of semen collection. Semen samples were produced by masturbation and collected into sterile containers. After complete liquefaction at room temperature, each sample was analysed using WHO guidelines. Semen was prepared for IUI by collecting a motile sperm fraction using either a swim-up procedure or discontinuous Percoll gradients. Samples were prepared on Percoll gradients if the sperm fraction using either a swim-up procedure or discontinuous Percoll gradients. Samples were prepared on Percoll gradients if the sperm concentration was ~0.5 cm below the uterine fundus. Luteal support was provided by administering 1000 IU HCG every 3 days for three doses after the insemination. If no menstrual period occurred, a quantitative β-HCG test was performed 16 days after insemination to determine the establishment of the biochemical pregnancy and clinical pregnancy was confirmed later by ultrasound scanning. In this study, a pregnancy was defined as one with the presence of an embryonic sac confirmed by ultrasound scanning.

**Collection of data**

Some standard demographic data were recorded such as age, body mass index [BMI; weight (kg)/height (m)^2] and pregnancy. If there was a doubling of LH values and no progesterone rise from baseline, the LH surge was taken as having occurred on the morning of the day blood was taken (i.e. 0800 h). If there was a doubling of the LH values and 1.5-fold increase in progesterone values over the basal value, the LH rise was taken as having occurred 6 h earlier (i.e. 0200 h). If there was a doubling of both the LH and progesterone values over the base value, the LH rise was taken as having occurred 12 h earlier (i.e. 2000 h previous day). All other variables such as date and time of HCG and IUI were calculated from the information recorded in the database.

**Statistical analysis**

Statistical analyses were performed using 'Instat', a statistical program by Graph Pad (San Diego, CA, USA). Pregnancy rate per cycle was compared using the Fisher’s exact test or the χ²-test, and in all cases P < 0.05 was considered significant. Analysis of variance (ANOVA) was used to compare differences of the mean age, BMI and cycles among the groups.

**Results**

A total of 463 couples who underwent 905 completed cycles of IUI between 1990 and 1995 was studied. The mean age of the female patients was 31.4 ± 0.3 years. The overall PR was 16% per cycle. Three scenarios in the temporal relationship of the LH surge, HCG administration and artificial insemination were identified, namely: (group A) subjects who had an endogenous LH surge but not given HCG; (group B) subjects who were given HCG after an observed LH surge; and (group C) subjects who were given HCG before the LH surge. The PR of the three groups and other results are presented in Table I. The highest PR was found in those patients in group B (20%), which was significantly higher when compared to group C (P = 0.04). The three groups were comparable with respect to age, cycle number and BMI (ANOVA).

In group A, 49 cycles (89%) were not stimulated with HMG (natural cycles) and resulted in four pregnancies (8.0%). HMG was used in the remaining 856 cycles (six cycles with HMG only from group A and 850 cycles with HMG and HCG from groups B and C) which resulted in 141 pregnancies (16%).

In group B, we calculated the time interval from the onset of the LH surge and the time of HCG injection to the time of IUI. The analysis showed that the timing of IUI based on the LH surge was not critical to PR within 3 days (Figure 1), but Figure 2 indicates that a higher PR was achieved if insemination was done within 48 h of HCG administration. Further analysis of the relationship between the interval between the LH surge and HCG administration and PR is shown in Figure 3. This
Table I. The mean (± SEM) age (years), body mass index (BMI, kg/m²), number of cycles (N), mean number of cycles per subject (n) and pregnancy rate per cycle in the three different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>N</th>
<th>n</th>
<th>Pregnancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: LH surge but no HCG</td>
<td>30.9 ± 5.0</td>
<td>23.8 ± 4.0</td>
<td>55</td>
<td>1.7 ± 1.1</td>
<td>9</td>
</tr>
<tr>
<td>B: HCG after LH surge</td>
<td>31.7 ± 5.0</td>
<td>25.3 ± 5.4</td>
<td>403</td>
<td>1.6 ± 0.8</td>
<td>20</td>
</tr>
<tr>
<td>C: HCG before LH surge</td>
<td>31.0 ± 4.6</td>
<td>24.8 ± 5.2</td>
<td>437</td>
<td>1.9 ± 0.9</td>
<td>14*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with group B.

LH = luteinizing hormone; HCG = human chorionic gonadotrophin.

Discussion

IUI combined with gonadotrophin stimulation has been advocated as an effective treatment for some types of infertility when compared with no stimulation (Martinez et al., 1991b; Chaffkin et al., 1991; Irianni et al., 1993). Whether this improvement in fecundity rates after HMG stimulation was related to the obtained polyovulation only or might have been caused by the correction of subtle ovulatory disorders still remains unclear. In this retrospective study, we looked at
certain parameters to make sure that they were not affecting
the results dramatically (e.g. mean age, mean BMI and mean
number of cycles), but certainly we did not and cannot look
at every possible confounding factor. Our overall PR was 16% per cycle, which is comparable with some past studies where
PR of 14–20 % have been achieved (Chaffkin et al., 1991;
Irianni et al., 1993). In our study, subjects who had natural
cycle IUI (group A) had requested the procedure either to
avoid gonadotrophin stimulation or because they had become
pregnant in the past on natural cycle IUI when this was
practised in the Unit. They are therefore a select group and
probably not comparable to groups B and C.

There has been considerable research into the timing of
ovulation. Past research has shown that, since there was a
wide variation in the time at which the LH peak occurred, the
onset of the LH surge was a more suitable reference point in
timing ovulation. Testart and Frydman (1982) in a large study
found that the time of follicular rupture was almost always
subsequent to the 37th hour after the onset of the LH surge.
Seibel et al. (1982) reported that human oocytes matured in
vitro reached metaphase II after 36 h and in vivo the oocyte
can take up to 38 h from the onset of the LH surge before
metaphase II was reached. The WHO in a multicentred,
collaborative study (WHO, 1980) found that ovulation may
occur from 24 to 56 h after the onset of the LH surge, with
a mean time of 32 h. Weinberg and Wilcox (1995) estimated
that mean viable lifetime in vivo for sperm was 1.4 days, while
the lifetime of the ovum appears to be less than a day. Moghissi
(1986) found that the fertilizing life span of the ovulated ovum
was up to 12 h after ovulation. Much of the variability in
timing for ovulation after the LH surge can be explained by
the varying definitions of the LH surge and different timing
methods. This may suggest that an oocyte can reach maturity
and retain the potential to be fertilized over a long fertilizing
window (mean 32 h ovulation time plus less than 24 h life span).

In our results, we observed the occurrence of spontaneous
LH surges in stimulated cycles to be 48%. The LH surge
occurred 78% of the time between day 9 and day 13 of the
cycle. We analysed the time interval from onset of the LH
surge to time of insemination in group B. There was no
significant change in pregnancy rates over these time intervals
(from 24 to 60 h). This time frame was in agreement with the
above-mentioned potential fertilizing window and held true
when HCG was given electively.

Several studies have stressed that an LH surge is a crucial
event for final maturation and ovulation (Tayor et al., 1995;
De-Koning, 1995). Martinez et al. (1991a,b) in their controlled
study of IUI suggested that the LH surge is a reflection of the
natural maturation process of the follicle. They also found
that the mean diameter of the pre-ovulatory follicles was
significantly smaller when early HCG injections were given
compared with cycles with the spontaneous LH surge. Accord-
ing to the general induction criteria, the HCG administration
usually took place earlier in the cycles when compared with
the occurrence of a spontaneous LH surge. Other data suggest
that early administration of HCG may have some detrimental
effects (Hillier et al., 1985; Tarin and Pelllicer, 1992). Casper
et al. (1988) studied a large number of in-vitro fertilization
cycles and found higher pregnancy rates where a spontaneous
LH surge occurred compared to the HCG-stimulated cycles
(28.8 vs 13.9%, \(P < 0.001\)). In our study, the results
supported the above findings: when HCG had been given after
the LH surge (i.e. allowing for full maturation of the follicle
to occur), a higher PR resulted (group B: 20% vs group C:
14%, \(P < 0.05\)). Further analysis in that group showed that
the timing of insemination based on the LH surge was not
critical to the achievement of pregnancy within 3 days, but if
it is based on the time of HCG administration a higher PR
may be achieved if insemination is performed within 2 days
(PR: 20 vs 9%; Figure 2). The other analysis within group B
found that the longer the time interval up to 20 h between the
LH surge and subsequent HCG administration the better the
PR up to 30–38% (\(P = 0.025\)). In group C, the PR appeared
to improve with the increasing interval between HCG and IUI
from <28 h up to 60 h (not significant). Although the trend
was very clear, the majority of cycles was in the time period
of 40–44 h and there were only 20% of the cycles earlier or
later than this time. Thus a proper testing of the relationship
can only be possible in a prospective trial, although the
relationship between LH surge (or HCG administration) and
ovulation would suggest that later IUI is more likely to be
synchronized with ovulation.

It has been our clinical practice to administer HCG to all
stimulated cycles even if a spontaneous LH surge occurred. It
may be questioned whether this practice is of value and a
randomized trial would be justified to prove any benefit
of HCG in this situation. It is also debatable as to whether elective
administration of HCG for ovulation induction is justified
given our results. The real reason for this practice is to enable
IUI to occur at a controlled time comparable with clinical and
laboratory convenience. Given the large window in which
inssemination can occur with good PR, there appears to be
little justification other than to reduce the amount of endocrine
monitoring required to detect the timing of an LH surge.

In conclusion, the highest PR was achieved in the group
where the LH surge occurred before HCG administration,
especially when HCG administration was delayed 8–20 h after
an observed LH surge: for those patients with an LH surge,
the timing of insemination based on the LH surge was not
critical to the achievement of pregnancy within 3 days, but if
based on the time of HCG administration, a higher PR was
achieved by inseminating within 48 h of HCG administration.

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