The use of urine LH detection kits to time intrauterine insemination with donor sperm

Ahmad F. Khattab, Fayez A. Mustafa, Philip J. Taylor

BACKGROUND: The study was carried out to determine the most likely time of day for the onset of the LH surge as detected using urine LH dipsticks, and to calculate the optimum time interval from the onset of the LH surge to intrauterine insemination (IUI). METHODS: A prospective study of 1540 cycles of IUI with donor sperm at Cleveland Fertility Centre, Middlesbrough, between June 1990 and February 2004. Only 951 cycles (where a positive urine LH dipstick result was immediately preceded by a negative result) were included in our study. To determine the best time interval between the onset of the LH surge and IUI, women were divided into five subgroups according to the positive urine test–IUI time interval and the pregnancy rate and live birth rate per cycle were calculated for each group. RESULTS: The first positive test was most frequently (44.5%) found at lunch-time (11:00–15:00). The live birth per cycle achieved was 5.6% when the insemination was performed 18–23 h from the first detection of the LH surge, and 11.7% when it was performed between 24 and 42 h. The live birth rate declined to 6.5% when IUI was performed later than that. Overall, no significant differences were discovered in live birth or pregnancy rate when insemination was performed at any of the time points between 18 and 53 h. CONCLUSION: Our study suggested that lunch-time is the best time to check for the LH surge using urine dipsticks and insemination at any time between 18 and 53 h after the onset of the surge will produce optimal results.

Key words: donor insemination/intrauterine insemination/timing of LH surge/urine LH dipsticks

Introduction

Traditional methods for timing insemination have used basal body temperature charts or cervical mucus assessment. Newer methods involve urine LH kits such as Clearplan (Unipath, Bedford, UK), which provide a convenient and reasonably reliable method (Vermesh et al., 1987; Robinson et al., 1992; Lashen et al., 1999). Four randomized control trials (RCT) compared these two methods (the traditional and the newer methods) of timing insemination (Barratt et al., 1989; Frederman et al., 1990; Odem et al., 1991; Robinson et al., 1992). Two of these trials used intracervical insemination while the other two were presumed to use intrauterine insemination but did not clearly say so. Meta-analysis of these trials (Flierman et al., 1997) showed no benefit from using the LH kits in terms of pregnancy rates per cycle. One study (Robinson et al., 1992) found a significant reduction in the number of patient visits per insemination cycle. Another study (Frederman et al., 1990) found the use of urinary LH dipsticks advantageous with regard to cost and time expenditure.

The advent of ICSI has reduced the demand for donor insemination (DI) (Human Fertilisation, Embryology Authority, 2000). ICSI is often preferred to DI because the resulting child is genetically related to both parents (Schover et al., 1996). Some couples choose DI primarily because they object to the IVF and ICSI or through fear of potential genetic risks with ICSI. Conversely when a couple has not achieved a successful pregnancy with ICSI, they may want to proceed to DI as an alternative treatment. However, the most common motivation for choosing DI was that it was cheaper than ICSI (Schover et al., 1996).

This paper determines the most likely time of day for the onset of the LH surge as detected in urine, and demonstrates the optimum time interval from the onset of the LH surge detected in this way to insemination.

Materials and methods

A total of 1540 inseminations with donor sperm was performed in 362 women aged 23–45 years at Cleveland Fertility Centre, Middlesbrough, UK from 17th June 1990 to 2nd February 2004. The internal review bureau approval was obtained. Clearplan urine LH kits (Unipath, Bedford, UK) were used to detect the LH surge in these women. We considered the urine dipstick test to be ‘positive’ if the colour intensity of the test line was similar to the control line or at least half as intense in colour. It was considered ‘negative’ if
no blue line was visible or if the test line was less than half as deep as the control line.

The first part of our study was prospective. Our patients were instructed to collect four samples of urine daily, early morning (04:00–10:00), lunch-time (11:00–15:00), tea-time (16:00–20:00) and bed-time (21:00–00:00) starting 2–3 days before the first likely date of onset of the LH surge, as predicted by the length of their menstrual cycle. Women with a 28 day cycle started collecting their urine on day 11, counting day 1 as the first day of bleeding. Women were instructed to test the lunch-time urine sample daily and were asked not to pass urine for 4 h prior to collecting the sample to be tested. The other samples were stored in a refrigerator and were only tested in retrospect if the lunch-time sample was positive. In that event they were first warmed to room temperature before testing. Otherwise they were merely discarded. In this way we were able to clearly define the onset of LH surge within a matter of hours.

In 951 cycles there was a correctly recorded positive urine LH test immediately preceded by a negative one. Only these cycles were included in our study. All women who failed to test the sample immediately preceding the positive test or if the urine test was not ‘clearly positive’ or ‘clearly negative’ at any time according to the criteria defined above were excluded from our study. No control group was included in this study.

In this study all inseminations were by the intratuterine method exclusively. The semen used contained a minimum of $2 \times 10^8$ motile sperm in the post-thaw sample. Just a single insemination was done in any one cycle. All inseminations were performed by two clinicians. The start date was the date when donor insemination was first performed. Any treatments performed prior to that date by ICI and any treatments previously performed elsewhere were disregarded. Thus the first cycle of IUI with donor sperm (IUID) at our centre was counted as treatment cycle no. 1 regardless of any previous treatment.

The second part of our study was retrospective. The IUI was done according to the convenience of the patients and the staff, mostly in the evening except weekends where it was done at any convenient time. The interval between the first positive urine test and IUI was between 18 and 53 h. This time interval was retrospectively divided into five groups and the pregnancy rate and live birth rate were calculated in each group.

**Statistical analysis**

We used stata 8, Texas, USA to analyse our data. $\chi^2$-Test and logistic regression model were used as appropriate. $P < 0.05$ was considered statistically significant.

**Results**

Out of 951 cycles included in the study, in 257 (27%) the first positive test was in the early morning sample. In 423 (44.5%), the first positive test was at lunch-time (most frequent). First positive test was at tea-time in 149 cycles (15.7%), and at night-time in 122 cycles (12.8%). These data are shown in Figure 1. We analysed these data using the $\chi^2$-analysis which is based on the null hypothesis. We assumed that the number of positive tests is equal in each category (237.75) as shown in Table I.

Performing a $\chi^2$-test (sum of $[(O – E)^2]/E$) on 4 – 1 = 3 degrees of freedom gives a strong indication to reject the null hypothesis: $\chi^2 = 235.4$, $P < 0.001$ ($O =$ observed number of events; $E =$ expected number of events). So there is no evidence to support the hypothesis that the number of positive tests is equal in each category. It is also clear that the largest number of first positive tests was found at lunch-time.

In our study, 132 pregnancies and 105 live births were achieved. These values make the overall pregnancy rate ~14% and the live birth rate 11%.

Cycles were divided into five groups according to the ‘first positive urine test–IUI time interval’. The pregnancy rate and live birth rate were calculated for each group. The number of cycles by time interval from first positive urine test to insemination, pregnancy rate, and live birth rate are shown in Table II. The pregnancy rate was 11.3% and the live birth rate 5.6% when the IUI–first positive urine test time interval was 18–23 h. Similar pregnancy and live birth rates were achieved (12.8 and 11.5% respectively) when the interval = 24–29 h. The equivalent values are 14.5 and 11.7% when the interval = 30–35 h. Odds ratio (OR) = 1.52 (95% CI 0.83, 2.79) ($P = 0.171$) for pregnancy rate; OR = 2.04 (95% CI 0.97, 4.31) ($P = 0.061$) for live birth rate. $^+$ = plus 0–59 minutes.

**Figure 1.** The percentage of cycles with first positive urine LH test by time. A $\chi^2$-test was performed to investigate whether the number of positive tests was uniformly distributed across the four time categories and this hypothesis was rejected at the level of $P < 0.001$.

![Figure 1](https://academic.oup.com/humrep/article-abstract/20/9/2542/2356829 by guest on 16 October 2018)

**Table I.** Observed and expected percentage of cycles by time of positive LH test

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Total</th>
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<td>4–10</td>
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<td>11–15</td>
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**Table II.** Pregnancies and live births per cycle by time interval from first positive urine LH dipstick test to insemination

<table>
<thead>
<tr>
<th>Time interval (h)</th>
<th>No. of cycles</th>
<th>Pregnancies</th>
<th>Live births</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–23+</td>
<td>53</td>
<td>6 (11.3)</td>
<td>3 (5.6)</td>
</tr>
<tr>
<td>24–29+</td>
<td>145</td>
<td>21 (14.5)</td>
<td>17 (11.7)</td>
</tr>
<tr>
<td>30–35+</td>
<td>460</td>
<td>59 (12.8)</td>
<td>53 (11.5)</td>
</tr>
<tr>
<td>36–42+</td>
<td>216</td>
<td>39 (18)</td>
<td>27 (12.5)</td>
</tr>
<tr>
<td>43–53</td>
<td>77</td>
<td>7 (9.1)</td>
<td>5 (6.5)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

The pregnancy rate = 11.3% and the live birth rate = 5.6% when the IUI–first positive urine test time interval = 18–23 h. The equivalent values are 14.5 and 11.7% when the interval = 24–29 h. Similar pregnancy and live birth rates were achieved (12.8 and 11.5% respectively) when the interval = 30–35 h. No significant difference was noted (18% pregnancy rate and 12.5% live birth rate) when the IUI was done 36–42 h from the first positive urine test. The pregnancy rate declined to 9.1% and live birth rate to 6.5% when the IUI was performed beyond 43 h. Odds ratio (OR) = 1.52 (95% CI 0.83, 2.79) ($P = 0.171$) for pregnancy rate; OR = 2.04 (95% CI 0.97, 4.31) ($P = 0.061$) for live birth rate. $^+$ = plus 0–59 minutes.

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was 18–23 h. The equivalent values were 14.5 and 11.7% when the interval was 24–29 h. Similar pregnancy and live birth rates were achieved (12.8 and 11.5% respectively) when the interval was 30–35 h. No significant difference was noted (18% pregnancy rate and 12.5% live birth rate) when the IUI was done 36–42 h from the first positive urine test. The pregnancy rate declined to 9.1% and live birth rate to 6.5% when the IUI was performed beyond that time interval.

The pregnancy data were put into a logistic regression model (Binomial distribution, Logit link function). This compared the number of pregnancies per cycle in the 24–42 time period against the number in the 18–23 and 43–53 h combined.

The resulting odds ratio (OR) was 1.52 with 95% confidence interval (CI) (0.83, 2.79) \( (P = 0.171) \). This result was not statistically significant. So there is insufficient evidence to support the hypothesis that the pregnancy rate was significantly higher when the IUI was performed 24–42 h after the first positive urine test.

The live birth data were put into a similar logistic regression model. As before, this compared the number of live births per cycle in the 24–42 time period against the number in the 18–23 and 43–53 h combined.

The OR was 2.04 with 95% CI (0.97, 4.31) \( P = 0.061 \).

So the odds of live birth per cycle were approximately doubled in the 24–42 interval compared to the baseline category (tail-ends). However, this result was also not statistically significant.

Although more pregnancies and live births were achieved when the first positive test–IUI time interval was 24–42 h, this difference was not statistically significant.

Discussion

Many clinical methods (such as day of the cycle, the quality of the cervical mucus, and temperature charts) have been used to provide a reference point for the timing of insemination in relation to ovulation.

Urine LH kits such as Clearplan provide a convenient and reasonably reliable method for timing of ovulation (Vermesh et al., 1987; Robinson et al., 1992; Lashen et al., 1999), and was the only method that we used in our study.

Ovulation is related in time to the onset of the LH surge, and occurs 40–45 h following the onset of this surge as detected in blood. The commonest time for the LH surge (as detected in blood) to commence is between 05:00 and 09:00. Repeated serum testing shows that 45% of LH surges commence at this time. LH is secreted in pulses, on average every 90 min. The half-life is 20–60 min. The serum levels therefore fluctuate considerably. The accurate determination of the LH peak by serum testing requires repeated and frequent blood sampling, which is not practical.

With the urinary assay of LH, these fluctuations are partially overcome by allowing the urine to accumulate for 3–4 h in the bladder prior to testing. The LH surge as detected in urine inevitably becomes apparent some hours after it can be detected by frequent blood tests. Additionally, the result may be affected by factors such as the fluid intake.

Despite these problems, urine kits can be used as frequently as necessary, whilst for blood samples this is not practical. In our view it is this factor that makes LH detection kits the most convenient option. By testing just once daily (at lunch-time), and then testing in retrospect the previously saved samples only when the noon sample is positive, we can define the onset of the LH surge within a matter of hours. We analysed the results of 951 spontaneous cycles included in our study. There were clearly more first positives in the lunch-time test. This difference in results is highly significant \( (P < 0.001) \) (Figure 1).

The results confirmed the tendency of the LH surge to start in the morning. However, an early morning sample of urine may well have been produced predominantly earlier in the night, and will most commonly test negative despite an LH surge that is already apparent on serum screening. A urine test done at lunch-time will detect >70% of LH surges that will start that day. We used just one urine LH kit daily and an additional two or three more for testing in retrospect only on the day of a positive result. The method, therefore, is a very cost-effective method of timing the onset of the LH surge in the clinical practice. This information has profound implications for anybody who uses a single daily LH dip-stick. Such practice, by testing the first sample on the day, will mostly only detect the LH surge ~1 day after it has started. When our women obtain a positive result they have always to test the previously saved samples in retrospect so that we can define more precisely when their surge started.

There is a window of time when insemination may be effective. If it is done too soon, the sperm may have lost their fertilizing capacity before the oocyte arrives; done too late, the oocyte will be too old for fertilization to occur (Wilcox et al., 1995). If intracervical insemination (ICI) is used, the window of time is likely to be shorter and earlier—as the cervical mucus is likely to be impenetrable well before the oocyte is too mature. This may explain the relatively poor results of ICI when used with frozen sperm, as has been shown by a systematic review (O’Brien and Vandekerckhove, 2000) of 12 RCT, which compared IUI with ICI using fresh and frozen sperm. The overall pregnancy rate per cycle was 18% in the IUI group versus 5% in the ICI group. When frozen semen was used, IUI significantly increased the pregnancy rate per cycle and per woman. However, no significant difference was found in IUI or ICI when fresh semen was used (Silva et al., 1989; Byrd et al., 1990; Patton et al., 1992; Peters et al., 1993; Williams et al., 1995; Mattoras et al., 1996; Goldberg et al., 1999; Carroll and Palmer, 2001).

In Table II it is clear that there is little variation in the results as indicated by pregnancy rate and live birth rate when the insemination was performed between 18 and 53 h from the onset of the LH surge detected by urine kits. Although the number of cycles in the first and last group was limited (53 and 77) compared with the number of cycles in the other three groups (145, 460, 216), we found no significant difference in the pregnancy rate and live birth rate per
cycle of insemination in the five groups, where the first positive urine test—IUI time interval was between 18 and 53 h [OR 1.52 with 95% CI (0.83, 2.79), P = 0.171 for pregnancy rate; OR 2.04 with 95% CI (0.97, 4.31), P = 0.061 for live birth rate].

Considering these results, we recommend that insemination should be done at any time between 15 and 53 h from the onset of the LH surge as detected in urine.

It is emphasized that these values refer to IUI when four samples of urine are collected daily starting 2–3 days before the expected date of ovulation. Obviously, if the onset of the surge is to be defined in this way it is essential to have a clearly negative result preceding the positive.

Centres that test only once daily cannot precisely define the onset of the surge. This error is compounded if they choose to do that one test, as they usually do, upon first rising. Such a system will, in a majority of cycles, fail to detect the onset of the surge until the day after it has started. If IUI is then done during the following day, many patients will be treated too late and the results will be poor. This is a common error. Although this work has never been done for intracervical insemination, it is likely that the optimum time interval is then less. Errors such as the above will then be further compounded.

It is also emphasized that these values refer only to the urinary detection of a spontaneous LH surge, and not to serum samples. It has been suggested (Horn et al., 1998) that serum testing for LH may be better, but the paper contained a number of faults, including those referred to above. The problem with serum testing is the need to attend for repeated sampling. Even then, there is usually a 24 h time interval between samples, so that it is not possible to define the onset of surge with any precision.

In conclusion, lunch-time (11:00–15:00) is the best time of the day to check for the LH surge using urine kits. Our results suggest that insemination at any time between 18 and 53 h from the first positive urine LH test will produce reasonable results.

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References


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