A new rapid and effective method for treatment of unexplained infertility

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BACKGROUND: Artificial insemination (intrauterine insemination by husband or artificial insemination by husband) is often tried as first treatment for couples with unexplained infertility. Pertubation has previously proved to increase the chance of achieving pregnancy for these couples. The effect of pertubation on fertility can be mechanical as well as anti-inflammatory by using a substance that inhibits phagocytosis of the spermatozoa. The objective of the study was to investigate the effect on pregnancy rate of pre-ovulatory pertubation with low-dose lignocaine during clomiphene citrate and insemination cycles for couples with unexplained infertility. METHODS: In a prospective, open study, the patients were randomized, the day before ovulation, during a clomiphene citrate stimulated cycle to either pertubation with low-dose local anaesthetic or no pertubation before insemination. RESULTS: A total of 130 cycles were studied, 67 of which were randomized to pre-ovulatory pertubation and 63 to no pertubation treatment. There were 14.9% ($n = 10$) clinical pregnancies in the pertubated group compared with 3.2% ($n = 2$) in the group without pre-ovulatory pertubation ($P < 0.05$). CONCLUSIONS: The pertubation treatment significantly enhanced the clinical pregnancy rate and was well tolerated. No complications were noted. The combined treatment of clomiphene citrate, pertubation and insemination can be used as a cost-effective, first-line treatment for couples with unexplained infertility.

Keywords: pertubation; tubal flushing; unexplained infertility; clomiphene citrate; intrauterine insemination

Introduction

Investigation of infertility is initiated after 1 year of attempts to achieve spontaneous pregnancy. During such an investigation, blood samples are taken concerning the basal hormone levels and the ovulatory function in the female. The male partner primarily only has a sperm count carried out. For women with dysmenorrhoea, a laparoscopy can be a part of the infertility investigation to evaluate any presence of endometriotic implants. For most infertile women in Sweden, Hysterosalpingo-Contrast-Sonography (HyCoSy) has gradually replaced the laparoscopy and peroperative tubal patency test. The consequence is that an unknown number of couples are diagnosed as having unexplained infertility, although endometriosis might be the cause. The explanation of the reduced fertility caused by minimal peritoneal endometriosis is still not clear (Hull, 1992; Halis and Arici, 2004). Treatments with GnRH gave initial hope but have only reduced pain and not improved fertility (Olive, 2004). There are several reports on intraperitoneal changes with increased amounts of leukocytes having an enhanced capacity of phagocytosing spermatozoa and thereby reducing fertility (Haney et al., 1983).

Usually, artificial insemination (intrauterine insemination—husband, artificial insemination—husband) is tried as first treatment for couples with unexplained infertility (Verhulst et al., 2006). Thus, insemination during three to four cycles can be offered while the couples are on the waiting list for IVF. IVF is usually offered as a second treatment option, but the most effective way of obtaining pregnancy for couples with unexplained infertility has not yet been clearly demonstrated (Guzick et al., 1998; Pandian et al., 2005). There are recent studies indicating that endometriosis has a negative impact on the outcome of IVF for this patient group (Halis and Arici, 2004). Ovulation stimulation and insemination have also shown poorer results for endometriosis patients than for couples with unexplained infertility (De Hondt et al., 2005). Compared with untreated couples in natural cycles, ovulation stimulation alone has not significantly improved fertility (Guzick et al., 1998).

Tubal flushing or pertubation has previously proved to be one way of increasing the chance of achieving pregnancy for couples with unexplained infertility and early stages of endometriosis (Johnson *et al.*, 2005). Pertubation treatment can be administered in an outpatient clinic and represents a less invasive treatment alternative (Edelstam *et al.*, 2001). The effect of pertubation on fertility can be mechanical as well as immunological, e.g. inhibited phagocytosis of spermatozoa (Edelstam *et al.*, 1998) and by affecting levels of peritoneal factors such as cytokines (Oak *et al.*, 1985; Agic *et al.*, 2006). HyCoSy is carried out in most infertility investigations and the adjuvant effect of this examination on achieving spontaneous pregnancy is clinically well documented (Johnson *et al.*, 2005).

The present study was carried out to investigate the effect on pregnancy rate of pertubation with lignocaine in a balanced salt solution the day before ovulation. Lignocaine was used with the purpose of further evaluating the inhibitory effect on sperm phagocytosis *in vivo* and the fertility adjuvant effect previously found.

In this randomized, prospective, open study, the patients were randomized to either pertubation with low-dose lignocaine or no pertubation before insemination.

Materials and Methods

Couples on the waiting list for IVF owing to at least 1 year of unexplained infertility were offered an opportunity to participate in the study. More than 300 couples were invited and 152 came for an initial appointment. One hundred and thirty couples accepted to be included and were randomized in the study (Fig. 1). A complete infertility investigation had been carried out before referral to the fertility unit. The female patients had normal functioning Fallopian tubes confirmed by HyCoSy. They did not achieve pregnancy after this initial diagnostic pertubation and tubal flushing. The women participating were <38 years and their cycles were within normal range (25–35 days). Normal ovulatory function and absence of bacterial vaginosis had been confirmed. During the infertility investigation, there was a negative Chlamydia test. The male partner had a normal sperm count within the past 5 years. Thus, the infertility investigation had not revealed any cause of the infertility.

There was a previous unsuccessful assisted reproduction treatment (ART) of 22% in the control and 19% in the pertubated group, respectively.

During a clomiphene citrate (50 mg on cycle day 5–9) stimulated cycle, the patients monitored their cycle by detecting the LH surge using test sticks in a urine sample. In addition to the natural LH surge or for ovulation induction to avoid treatment during weekends when 1–2 follicles was ≥18 mm, 5000 IU HCG (Pregyl, Organon) was administered s.c. The pregnancy rate is the same when LH surge or HCG is used for ovulation induction (Deaton *et al.*, 1997). In the non pertubated group there was 63 patients of which 32 (51%) received HCG injection before the LH surge. In the pertubated group the corresponding figures were 67 and 39 (58%). For the pertubation treatments, a batch of special solutions was produced with 1.73 mM lignocaine hydrochloride in a balanced salt solution (expiry date July 2006). The patients were randomized on the pre-ovulatory day to pertubation or no pertubation. For randomization, sealed and numbered envelopes were prepared externally and used consecutively. A vaginal ultrasound examination was performed on the pre-ovulatory day to confirm a maximum of two follicles ≥16 mm. The pertubation procedure (Fig. 2) was carried out in the same way as regular HyCoSy: again confirming absence of bacterial vaginosis, cleaning the portio surface with saline swab, then inserting and cuffing a hydrosonography catheter in the cervical canal. Blood pressure and heart rate were measured before and after pertubation.

After swim-up preparation, artificial insemination was carried out with $>1 \times 10^6$ motile spermatozoa 24–32 h after LH signal or given HCG injection (Ombelet *et al.*, 2003). For patients included in the per protocol (PP) analysis, the total number of motile spermatozoa after preparation was $>4 \times 10^6$. The outcome was measured as the clinical pregnancy rate with ultrasound-verified pregnancy. Only one complete treatment cycle was offered to each couple. Serum progesterone was checked 1 week after ovulation and if below luteal phase level, the cycle was not considered complete and a new treatment was offered in the next cycle.

All cycles with >2 follicles ≥ 16 mm were cancelled and a repeated attempt to treat was offered in a natural cycle. Also in cases of bacterial vaginosis before pertubation treatment, the cycle was cancelled and a new clomiphene citrate and insemination attempt was made after pharmacological treatment and cure.
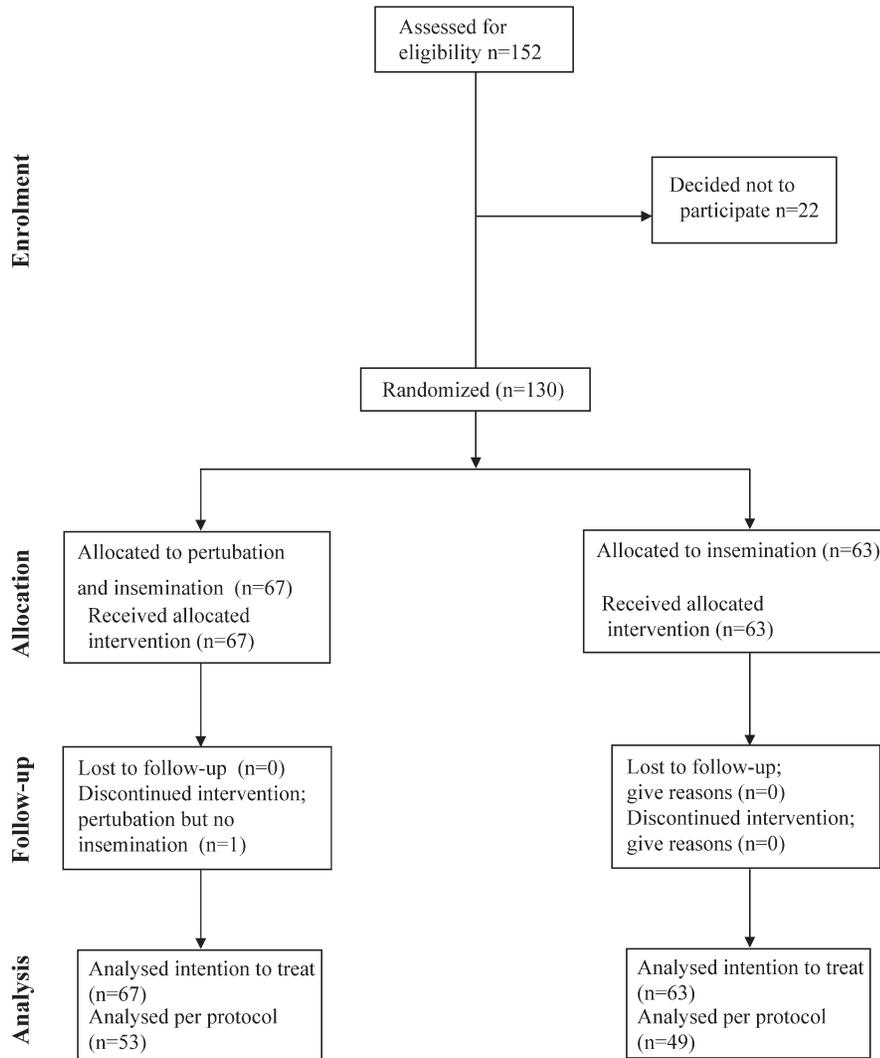
Statistics

For the calculation of significance of the primary outcome parameter, chi-square tests for the comparison of two proportions from independent samples were used. The number of patients needed for significance was calculated assuming a pregnancy rate of 16% in the control group and a pregnancy rate of 30% in a lignocaine-pertubation group, based on earlier clinical study results (Edelstam *et al.*, 2001). The calculations were based on chi-square approximation. The number of participating patients obtained reached a power of 65%. A value of $P < 0.05$ was considered significant.
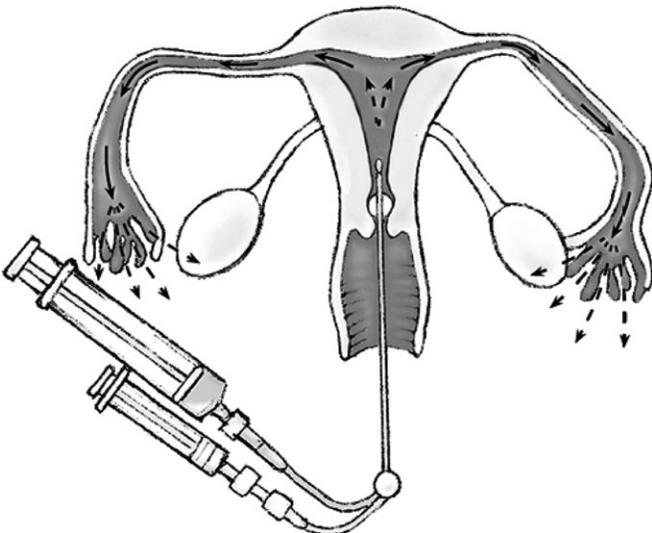
Results

A total of 130 couples were randomized and treated in the study. One complete cycle was offered to each couple. Of these cycles, 67 were randomized to clomiphene citrate and insemination cycles with pre-ovulatory pertubation. The control cases were 63 couples randomized to no-pertubation treatment during the clomiphene citrate and insemination cycle. There were no significant differences between the control and the pertubation group in the number of motile spermatozoa retrieved after preparation. In neither the intention-to-treat (ITT) group nor the PP group were there any significant differences in the subgroup of patients who achieved pregnancy. One insemination after pertubation was cancelled due to the male partner having a high temperature and being unable to deliver the sperm sample. Three midwives and seven gynaecologists participated in carrying out the inseminations ($n = 129$). There were no significant differences in clinical result rates between midwives and gynaecologists.

There were 14.9% ($n = 10$) clinical pregnancies in the pertubated group compared with 3.2% ($n = 2$) in the group without pre-ovulatory pertubation (ITT, Table I). In the pertubation group, one patient had a late spontaneous abortion after 21 complete pregnancy weeks whereas the remaining pregnancies ended with healthy born babies and a take-home baby rate of 14% compared with 3% in the control group. The increase in pregnancy rate following pre-ovulatory pertubation was statistically significant ($P < 0.05$). After PP analysis (i.e. sperm count after preparation $\geq 4 \times 10^6$ motile spermatozoa and

Assessed for
eligibility n=152

Decided not to
participate n=22

Randomized (n=130)

Allocated to pertubation
and insemination (n=67)
  Received allocated
  intervention (n=67)

Allocated to insemination (n=63)
  Received allocated
  intervention (n=63)

Lost to follow-up (n=0)
Discontinued intervention;
pertubation but no
insemination (n=1)

Lost to follow-up;
give reasons (n=0)
Discontinued intervention;
give reasons (n=0)

Analysed intention to treat
(n=67)
Analysed per protocol
(n=53)

Analysed intention to treat
(n=63)
Analysed per protocol
(n=49)

Figure 1: CONSORT statement flow diagram

Figure 2: Pertubation treatment carried out via a catheter with cuff in
the cervix canal

verified ovulation), the results were more obvious with 18.9%
clinical pregnancies after pertubation ($n = 53$) and 4.1% if no
pertubation ($n = 49$) treatment was given ($P < 0.05$)
(Table I). The Number Needed to Treat (NNT) was 8.51 with
confidence interval ± 0.83, i.e. NNT of nine patients. The
choice of pretreatment with the low, single-dose clomiphene
citrate of 50 mg resulted in only singletons and no twin
pregnancies. Finally, there were no significant differences in
blood pressure or heart rate before and after pertubation (data
not shown).

There were no demographic differences between the groups.
The female mean age was 33 years in both groups. In addition,
there were no significant differences in body mass index or
mean duration of infertility. In the pertubated group, the
mean infertility duration was 2.5 years and in the non-
pertubated group, 2.4 years. The general demography of the
patients is tabulated in Table I. The mean number of follicles
during the treated cycles did not differ significantly, with 1.4
in the non-pertubated group compared with 1.3 for those who
received pertubation. Neither did the mean number of follicles
differ significantly between treatments. There were no

Table I. Demography, treatment and outcome for patients with unexplained infertility in the trial of pertubation (low-dose lignocaine) versus no pertubation during a stimulated cycle for artificial insemination.

Parameter	Pertubated	Not pertubated	*P*-value
N (total)	67	63	N.A.
Age (years)	32.7 ± 3.1, CI 32.0–33.4	32.8 ± 3.3, CI 32.0–33.6	0.93
Weight (kg)	61.8 ± 10.3, CI 59.3–64.3	62.1 ± 9.9, CI 59.8–64.5	0.92
Height (cm)	166.8 ± 7.1, CI 165.1–168.5	166.5 ± 5.8, CI 165.1–167.9	0.72
Duration of infertility (years)	2.5 ± 1.1, CI 2.2–2.8	2.4 ± 1.0, CI 2.2–2.6	0.76
No. of follicles >16 mm	1.27 ± 0.45, CI 1.16–1.38	1.43 ± 0.7, CI 1.26–1.60	0.13
Clinical pregnancy *n* (%) ITT[1]	10 (14.9%), *n* = 67	2 (3.2%), *n* = 63	**0.044**
Clinical pregnancy *n* (%) PP[2]	10 (18.9%), *n* = 53	2 (4.1%), *n* = 49	**0.045**

Values are mean ± SD.
[1]Intention to treat.
[2]Per Protocol, i.e. total number of motile spermatozoa after preparation >4 10^6 and luteal phase S-Progesterone positive.
CI, confidence interval.

significant differences between the control and the pertubation group in the number of motile spermatozoa retrieved after preparation. In neither the ITT nor the PP group were there any significant differences in sperm motility in the subgroup of patients who achieved pregnancy. In addition, no age-dependent results of the treatment were found.

Discussion

Ovulation stimulation or intrauterine insemination is a fertility treatment with a lower pregnancy rate per cycle compared with IVF. If a completed investigation for infertility has not revealed any cause of infertility, clomiphene citrate and insemination is often tried as the first-line treatment. Insemination can be carried out while the couple is on the waiting list for IVF. Clomiphene citrate and insemination has an average pregnancy rate of 8% per treatment cycle (Guzick *et al.*, 1998). With an increasing number of infertile years, the pregnancy rate per treatment cycle will be gradually reduced due to attenuation bias (Hull, 1992). The success rate with clomiphene citrate and insemination cycles is thus substantially lower than the overall approximately 35% pregnancy rate per IVF cycle. However, insemination is less invasive and can be carried out at a substantially lower cost. This investigation has focused on evaluating the adjuvant effect of pertubation on the insemination success rate.

For couples undergoing an infertility investigation, HyCoSy is carried out in most cases. The procedure consists of a tubal flushing and results in an increased pregnancy rate during the following months (Johnson *et al.*, 2005). When hystero-salpingo-graphy (HSG) was performed as an X-ray, there were two different contrast options: an oil-based medium or a water-soluble medium. The pregnancy rate after using the oil-based contrast medium has resulted in twice as many pregnancies compared with a water-soluble medium (Rasmussen *et al.*, 1991). In addition, *in vitro* studies of the oil-based contrast medium lipiodol have shown a reduction of sperm phagocytosis (Mikulska *et al.*, 1994). Tubal flushing with lipiodol has been tried in a small, randomized clinical study on women with endometriosis and no spontaneous pregnancy for 3 years (Nugent *et al.*, 2002). After pertubation with lipiodol, 30% of the patients

achieved pregnancy compared with none of the patients randomized to expectancy. However, there are occasional reports of serious complications after HSG using an oil-based contrast, presumably due to fat embolism (Uzun *et al.*, 2004). Compared with an oil-based contrast medium which can remain intraperitoneally (Miyamoto *et al.*, 1995), a local anaesthetic such as lignocaine is a well-tolerated drug and has the same capacity *in vitro* to reduce sperm phagocytosis (Edelstam *et al.*, 1998). Pertubation with lignocaine (0.1 mg/ml) in a balanced salt solution was tried in a previous clinical study with the same overall pregnancy rate as was described with lipiodol (Edelstam *et al.*, 2001). No complications were noted with low-dose lignocaine pertubations and no reproductive toxicological problems have been described (Ramazzotto *et al.*, 1985).

The present study has indicated a new treatment concept based on previously observed increased fertility after HSG. The effect of adding tubal flushing/pertubation during an insemination cycle is presumed to have two effects: mechanical and immunological. The mechanical effect is thought to be, e.g. opening of loose adhesions around the fimbriae (Rasmussen *et al.*, 1991). The *in vitro* observed reduced sperm phagocytosis is presumed also to have an immunological effect *in vivo* by enhancing the survival rate of spermatozoa (Edelstam *et al.*, 1998; Nugent *et al.*, 2002).

The patients who did not become pregnant after the tubal patency test (HSG/HyCoSy) in the infertility investigation were invited to participate in this study. Thus, all patients in the present study had an unexplained infertility of average 2.5 years and all were treated with clomiphene citrate and insemination. There were no significant demographic differences between those who, in addition, were randomized to obtain a pertubation with lignocaine in a balanced salt solution. There was a substantial and significant increase of 4.65 times more clinical pregnancies in the pertubated group. The relatively low pregnancy rate in the non-pertubated group is similar to that in previous studies with insemination after low-dose clomiphene citrate of 50 mg (Deaton *et al.*, 1997). It is also explained by the long, average infertility duration of 2.5 years and thus a result of attenuation bias (Hull, 1992). Another important factor is the relatively high average age of 33 years. Further, the average infertility of >2 years and the previous

unsuccessful ART of 22% and 19%, respectively, in each group explain the low background success rate in the control group.

The fact that only the lowest dose of 50 mg of clomiphene citrate was used resulted in only singletons and no twin pregnancies. There were no differences in the number of follicles ≥16 mm, so the differences between the treatment groups were not due to intense ovulation stimulation. The combination of using low-dose clomiphene citrate for patients with a long duration of infertility and not using positive urinary HCG as an effect parameter, but only considering ultrasound-verified clinical pregnancies, explains the relatively low pregnancy rate in the control group.

Conclusions

Pertubation treatment was very effective in significantly enhancing the clinical pregnancy rate in clomiphene citrate and insemination cycles. The absolute increase in pregnancy rate was 11.7% and the relative increase in pregnancy rate was 465% or 4.5 times. Pertubation with low-dose lignocaine solution was well tolerated. The pertubation treatment used in this study proved to be a safe treatment option without complications. The combined treatment of clomiphene citrate, pertubation and insemination can be used as the first-line treatment for couples with unexplained infertility.

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