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Ultrasound-guided artificial insemination: a randomized controlled trial

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BACKGROUND: The aim was to ascertain whether using ultrasound guidance during intrauterine insemination (IUI) could increase pregnancy rates (PRs).

METHODS: The population under study consisted of 73 consecutive couples subjected to IUI in our Human Reproduction Unit, between June and December 2006, with a total of 231 IUI cycles performed. The patients were randomized using a computer-generated random numeric table into two groups: ultrasound-guided IUI group ($n = 33$) and clinical IUI group ($n = 40$).

RESULTS: The PR was 16.0% per cycle in ultrasound-guided IUI and 16.8% in the control group, no statistically significant differences being observed between the groups. The 95% confidence interval for the difference in PRs of 0.8% was −8.8 to 10. There were no differences in PR per woman, nor in first-cycle PR. The cumulative PR was also similar in both populations. Although the initial intention was to perform a study involving a larger number of cases, after a first interim analysis, the study was interrupted due to its futility. There were no differences in PR according to the different cervico-uterine angles.

CONCLUSIONS: Ultrasound-guided IUI does not produce better results than blind insemination, because the PR per cycle is similar. ClinicalTrials.gov ID NCT00809952.

Key words: intrauterine insemination / pregnancy rate / ultrasound

Introduction

Intrauterine insemination (IUI) is currently the primary therapeutic modality for unexplained infertility and for infertility caused by mild to moderate female and male pathologies, with typical per cycle pregnancy rates (PRs) ranging from 10 to 20%. The success of IUI depends on a number of parameters linked both to the pathology underlying the infertility and to the treatment. The majority of published studies on IUI focus on ovarian stimulation and sperm management, whereas insemination techniques have received scant attention. In *in vitro* fertilization (IVF), however, embryo transfer technique, and specifically ultrasound-guided transfer, has received increasing attention in recent years. The use of ultrasound-guided embryo transfer facilitates atraumatic embryo placement (Matorras *et al.*, 2002, 2004), and it has been reported that the use of abdominal ultrasound during transfer produces higher PRs compared with transfer based purely on clinical methods (Coroleu *et al.*, 2000; Sallam and Sadek, 2003). Ultrasound allows us to visualize the cervico-uterine angle, reducing the number of difficult cervical catheterizations (Sallam and Sadek, 2003) as well as cervical manipulations. Ultrasound visualization of the endometrial cavity prevents the catheter from impacting the uterine fundus. Cervical and/or endometrial manipulations increase uterine contractions due to the secretion of prostaglandins and/or oxytocin (Dorn *et al.*, 1999; Lesny *et al.*, 1999), and expulsion of >40% of the volume introduced into the uterine cavity has been reported (Knutzen *et al.*, 1992; Mansour *et al.*, 1994).

One of the variables that influence the outcome of IUI is the number of inseminated spermatozoa (Burr *et al.*, 1996; Wainer *et al.*, 2004). If uterine contractions are present, almost half of the introduced volume may be expelled, thus reducing the number of spermatozoa with access to the tubes and therefore the success of the procedure.

Ultrasound visualization of the catheter makes it possible to deposit the prepared sperm without touching the uterine fundus, thus avoiding uterine contractions.

The objective of this study was to determine the potential benefits of using abdominal ultrasound during IUI, by comparing the PRs of ultrasound-guided with non-ultrasound-guided inseminations.

Materials and Methods

The sample under study consisted of 73 consecutive couples subjected to IUI in our Human Reproduction Unit, between June and December 2006, with a total of 231 IUI cycles performed. All couples were subjected to the infertility work-up as follows: transvaginal ultrasonography, basal hormone tests (third day of the cycle), hysterosalpingography and sperm analysis. The woman's inclusion criteria in our IUI program were at least one patent tube, normal cavity, basal follicle stimulating hormone (FSH) $<$ 10 mU/ml and age under 40 years. IUI with husband's sperm (IUI-H) was indicated when, after sperm preparation, at least 5 million motile spermatozoa were recovered; this was performed in 58 cases (79.45%). In the remaining 15 cases (20.55%), IUI was performed with donor sperm (IUI-D) either because of azoospermia ($n = 6$), failure to recover spermatozoa during testicular biopsy ($n = 5$), or in women without a male partner ($n = 4$). No previous infertility treatments had been carried out.

The stimulation protocol was the same for all patients, consisting of a daily subcutaneous injection of 150 IU of recombinant FSH (Gonal F®, Serono Laboratories, Spain), started on Day 2 of the menstrual cycle. The ovarian response to stimulation was regulated by adjusting the dose according to transvaginal folliculometry and plasma estradiol assay, as previously reported (Matorras *et al*., 1995, 1997). When at least one follicle had a diameter of 16–20 mm, we administered one subcutaneous dose of 0.25 mg/day of cetrorelix (Cetrotide®, Serono Laboratories), maintaining this dose until the day on which hCG was administered.

From the start of antagonist administration, we added 75 IU/day of recombinant LH to the FSH stimulation protocol (Luveris®, Serono Laboratories). We administered 250 mcg of rHCG (Ovitrelle, Serono Laboratories) when there were at least two follicles with a diameter of $\geq$18 mm, with estradiol level $\geq$600 pg/ml ($\geq$2200 pmol/l). The treatment cycle was cancelled if there were $<$2 or $>$5 follicles. We carried out one single insemination per cycle at 37–39 h after hCG injection, with a maximum of six insemination cycles per patient (Matorras *et al*., 2000; Osuna *et al*., 2004).

All the semen samples were prepared with density gradients of Puresperm (NidaCom Laboratories, Sweden) as described previously (Matorras *et al*., 2000, 2003, 2006). The luteal phase was supplemented with vaginal micronized progesterone (Utrogestan®, Besins-Iscovesco Laboratories, Paris, France) at doses of 200 mg every 12 h. Institutional Board approval and informed consent were obtained.

Patients were randomized using a computer-generated random numeric table and the numbers entered into closed envelopes. Group I ($n = 33$) corresponded to ultrasound-guided insemination and Group II ($n = 40$) to insemination using clinical criteria. After randomization there were no women who refused to participate in the study. In both groups, the patients attended the appointment with a full bladder, and the same catheter model was used for the procedure in both instances: the Frydman Soft model (Prodimed, France), which has two channels: rigid outer (with a cap) and flexible inner; all IUIs (both ultrasound IUI and clinical IUI) were performed by staff gynaecologists and the majority (90%) were carried out by the same gynaecologist (O.R.).

In patients undergoing ultrasound-guided insemination, prior to insemination, the cervico-uterine angle (the angle formed between a line joining the external and internal cervical orifices and another line joining the internal cervical orifice to the uterine fundus) was measured on a longitudinal uterine image, obtained via abdominal ultrasound. Patients were then assigned to one of the following four categories: (i) no angle or narrow angle ($<30^\circ$); (ii) moderate angle ($30–60^\circ$); (iii) wide angle ($>60^\circ$) and (iv) retroflexed uterus.

In patients undergoing ultrasound-guided insemination, the external (rigid) sheath of the catheter was moulded in advance according to the angle, and introduced into the cervical cavity to 1 cm past the internal cervical orifice. Via this sheath, under abdominal ultrasound guidance, we introduced the flexible inner catheter into the uterine cavity, until the tip was located at a distance of 1 cm from the fundus. Abdominal ultrasound was performed by mean of a General Electric Medical Systems Logic 3 ultrasound machine with the 3.5C abdominal probe.

In patients undergoing non-ultrasound-guided insemination, cervical catheterization was performed, until the resistance of the internal cervical orifice was passed, introducing the internal catheter according to clinical criteria, based on a hysterometry carried out during the infertility work-up. In no cases, was cervical tenaculum or hysterometer needed at the time of the procedure (either in ultrasound IUI or in classical IUI). Pregnancy was defined by visualization of the gestational sac at vaginal ultrasound 3–4 weeks after insemination.

For statistical analysis, we used the χ^2 and ANOVA tests, following the standard applicability criteria. The Statistical Package for Social Science (SPSS) version 13.0 was used. Alpha value was defined as $P < 0.05$. Cumulative PR was calculated according to Cramer *et al*. (1979).

Sample size predetermination was calculated as follows: assuming a per cycle PR of 12% (on the basis of the Spanish Insemination Register results) (Hernandez *et al*., 2006), an alpha of 0.05 and a 0.20 β risk in a bilateral contrast; 551 cycles in each group would be needed to detect a 6% difference between the groups, employing the arcsin calculation method. Intermediate cut-offs for analysis were defined at 20, 50 and 80% of the estimated sample size, to eventually redefine the size of the population.

Results

In the first cut-off, a total of 231 stimulated cycles in 73 patients were included. In 58 patients, the sperm sample was the husband's sperm (190 cycles) and in 15 patients donor sperm was used (41 cycles). In the ultrasound-guided group, 106 cycles were carried out and the in non-ultrasound-guided group, 125 cycles. The main characteristics of the ultrasound-guided IUI and classical IUI populations were similar with regard to age, hormonal status, ovarian stimulation and sperm parameters (Table I). The number of women completing six cycles of treatment was similar in both groups: seven in ultrasound-guided IUI (seven with husband's sperm and zero donor) and nine in classical IUI (seven with husband's sperm and two donor).

The PR per cycle was 16.0% (17/106) in the ultrasound-guided group, very similar to the 16.8% (21/125) in the classical IUI group (Table II); 95% confidence interval for the difference in PRs was 0.8% (−8.8/10), $P = 0.8763$. There were no differences in PR per woman, nor in first-cycle PR between both groups. The cumulative PR was also similar in both populations (Fig. 1). There were no differences in the PR between ultrasound and non-ultrasound groups when analyzed according to the indication for IUI. Since the PR was essentially the same, the study was interrupted after recruitment and analysis of these 73 patients, who represented 20% of the estimated required number of cycles.

Miscarriage rate was similar in both groups, as was multiple PR. High-order multiple pregnancy was also similar (two triplets in the ultrasound-guided group versus one triplet in the non-ultrasound-guided group).

Table I General characteristics of ultrasound-guided IUI and classical IUI patients

	Ultrasound-guided IUI	Non-ultrasound-guided IUI
Number of patients	33	40
Number of cycles	106	125
Age	34.7 ± 2.7	35.1 ± 2.8
Body mass index	23.8 ± 2.8	23.7 ± 2.8
Basal FSH	8.2 ± 2.2	8.2 ± 2.6
Total FSH-r used	1339 ± 499	1382 ± 457
Estradiol on day of hCG	927 ± 48/4	1034 ± 464
Number of follicles >16 mm	3 ± 1.4	3.1 ± 1.4
Total motile sperm count after Pure-sperm (millions)	12.8 ± 6.2	14.1 ± 6.4
Idiopatic infertility (%)	27.3	27.5
Mild male factor (%)	51.5	52.5
% donor sperm	21.2	20

All differences were non-significant.

Table II PRs of ultrasound-guided IUI and classical IUI patients

	Ultrasound-guided IUI	Non-ultrasound-guided IUI	95% CI for differences
Per cycle PR	16.0 (17/106)	16.8 (21/125)	0.8 (−8.8/10)
First cycle PR	15.1 (5/33)	25.0 (10/40)	9.8 (−8.3/28)
Cumulative PR	80.7	74.0	
Per woman PR	51.5 (17/33)	52.5 (21/40)	1 (−24/22)
Multiple PR	17.6 (3/17)	14.3 (3/21)	−3.3 (−38/35)
Abortion rate	17.6 (3/17)	19.0 (4/21)	1.4 (−19/21)

Non-significant differences.

The ultrasound measurement performed before IUI showed a cervico-uterine angle between 30 and 60° in 57.7% of cases. After insertion of the speculum, the cervico-uterine angle was between 30 and 60° in 69.8% of cases, with a consequent decrease in the proportions of all the remaining cervico-uterine angle groups. There were no differences in PR according to the different cervico-uterine angles.

In the 97 cycles (including both ultrasound-guided IUI and classical IUI) where the cervico-uterine angle was recorded, the PR was similar according to the different angles. The PR was 9% when the angle was <30° (2/15); 21.5% when it was 30–60° (15/66); 25% when it was 60–90° (1/4) and 0% in retroverted uterus (0/12) ($P > 0.05$).

Discussion

IUI is a widespread technique requiring cervical catheterization to access the uterus, similar to embryo transfer in IVF. However, although in recent years, a number of authors have studied the different conditions associated with embryo transfer prognosis, the effect of IUI insemination technique has received little attention. Theoretically, IUI performed under ultrasound guidance could be associated with increased PR, since this may reduce cervical and endometrial damage as well as bleeding, thus reducing the release of prostaglandins as well as uterine contractions.

The use of ultrasonography as a support for assisted reproduction techniques has been widely used in IVF. Embryo transfer is usually associated with higher PRs, although no differences have been reported in a recent work (Drakeley *et al*., 2008). Difficulty of transfer, presence of blood in the catheter and uterine contractions all reduce implantation rates (Alvero *et al*., 2003). The use of abdominal ultrasound during transfer provides direct visualization of the movement of the catheter inside the endometrial cavity, reducing the frequency of difficult transfers and minimizing endometrial damage. However, its use in IUI has not been tested. Both IUI and embryo transfer require cervical catheterization and deposition of the end product of the reproductive technique inside the endometrial cavity.

IUI results are influenced by a number of parameters: female and male conditions, sperm characteristics and preparation, infertility status, ovarian stimulation and methodology, as has been demonstrated in a number of studies (Matorras *et al*., 1995; Osuna *et al*., 2004). However, the methodology of IUI technique has received scarce attention.

In IUI, the processed specimen is deposited using a blind technique, dependent on the skill of the physician. In IVF, it has been shown that blind transfer leads to inadvertent contact of the tip of the catheter

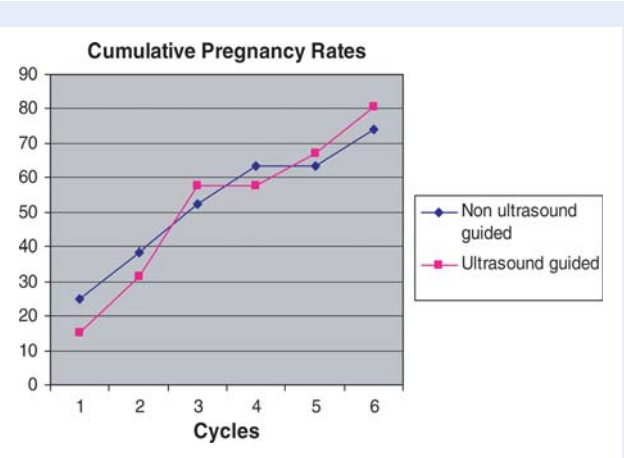

Figure 1 Cumulative PRs in ultrasound-guided IUI and in classical IUI.

with the uterine fundus in 17.4% of cases (Woolcott and Stanger, 1997), provoking an increase in uterine contractions (Lesny *et al.*, 1998); expulsion of >40% of the volume introduced into the uterine cavity has been reported (Harper *et al.*, 1961; Mansour *et al.*, 1994). Another cause of uterine contractions is the handling of the cervix in achieving catheterization. In IVF, ultrasound visualization of the cervico-uterine angle allows the catheter to be adapted to the angle, thus avoiding excessive manipulations and resulting in atraumatic catheterization. Rapid transfer also increases the chance of pregnancy in IVF (Matorras *et al.*, 2004). One of the prognostic factors for IUI is the number of motile spermatozoa deposited in the uterus. The presence of contractions may eliminate almost half of the spermatozoa, unbeknown to the clinician, and thereby limiting the results (Wainer *et al.*, 2004). The object of our study was therefore to ascertain whether using ultrasound guidance during IUI could increase the PRs. The control and study populations proved to be comparable regarding the main demographic and clinical parameters.

However, our study failed to find any differences between ultrasound-guided IUI and conventional IUI. In fact, PRs were very similar in the two groups, with regard to both PR per cycle (16.0 and 16.8%) and PR per woman (51.5 and 52.5%). Two explanations can be given. First of all, it is possible that filling the bladder alone could have the same insemination-facilitating effect as ultrasound-guided IUI insemination with a full bladder. In a number of the studies regarding ultrasound-guided embryo transfer, a full bladder was not required in the control group. The hypothesis of the insemination-facilitating effect of a full bladder seems to be supported by the fact that cervical manipulation was not required in any of the classical IUI cases.

On the other hand, the processes that follow the release of embryo/spermatozoa into the uterine cavity differ between IVF and IUI. In IVF, it has been reported that it is important to deposit the embryos in a specific place in the uterine cavity (Lewin *et al.*, 1997; Coroleu *et al.*, 2002), which is in many cases close to the site of implantation. However, in IUI, spermatozoa must still ascend through the fallopian tube, as far as the ampullary portion, where fertilization occurs. In IVF, cervical and endometrial damage could interfere with implantation, either directly or via prostaglandin release. However, in IUI, since implantation occurs approximately 7 days after insemination, cervical and endometrial damage are likely to have been repaired by this time.

The stimulation protocol employed during the study is more aggressive than is usual, and was associated with a high rate of high-order multiple pregnancy. In our unit, this has now been replaced by a lower starting dose of FSH.

In our study, we performed an intermediate analysis of results to redefine the sample size of the population, if necessary. Such analyses are relatively common when deciding whether to discontinue a study if clearly significant differences have been found with a smaller sample size than initially calculated. They are also useful for recalculating the size of the required sample if the observed differences are smaller than expected and a larger population is judged necessary. In our case, the differences in results were not only much lower than expected, but were practically non-existent. Only a 0.8% difference in per cycle PR was observed, and in favor of the non-ultrasound group, consequently the study was interrupted due to its futility.

With regard to the cervico-uterine angle, the majority of patients displayed an angle between 30 and 60°, especially after speculum insertion. In our experience, the cervico-uterine angle was not a prognostic factor, contrary to results published previously in IVF (Sallam *et al.*, 2002).

According to our experience, the use of ultrasonography during IUI complicates the technique, requiring an ultrasound probe and specialized personnel (ultrasound technician) and increases the amount of time required while obtaining similar results to the control group of patients for whom ultrasonography was not used. We consider atraumatic catheterization necessary for preventing uterine contractions, but this can be achieved both by requesting a moderately full bladder in order to reduce the cervico-uterine angle and by moulding the catheter to an angle of 30–60°, which corresponds to the angle observed in 70% of all patients. However, in our opinion, ultrasound-guided IUI should be recommended in cases where problematic cervical catheterization is expected.

In conclusion, our results indicate that ultrasound-guided IUI does not produce better results than blind insemination, since the PR per cycle is similar. Thus, we do not recommend the systematic use of ultrasound guidance during IUI.

References

Alvero R, Hearns-Stokes RM, Catherino WH, Leondires MP, Segars JH. The presence of blood in the transfer catheter negatively influences outcome at embryo transfer. *Hum Reprod* 2003;**9**:1848–1852.

Burr RW, Siegberg R, Flaherty SP, Wang XJ, Matthews CD. The influence of sperm morphology and the number of motile sperm inseminated on the outcome of intrauterine insemination combined with mild ovarian stimulation. *Fertil Steril* 1996;**65**:127–132.

Coroleu B, Carreras O, Veiga A, Martell A, Martínez F, Belil I *et al.* Embryo transfer under ultrasound guidance improves pregnancy rates after in-vitro fertilization. *Hum Reprod* 2000;**3**:616–620.

Coroleu B, Barri PN, Carreras O, Martinez F, Parriego M, Hereter L, Parera N *et al.* The influence of the depth of embryo replacement into the uterine cavity on implantation rates after IVF: a controlled, ultrasound-guided study. *Hum Reprod* 2002;**2**:341–346.

Cramer DW, Walker AM, Schiff I. Statistical methods in evaluating the outcome of infertility therapy. *Fertil Steril* 1979;**32**:80–86.

Dorn C, Reinsberg J, Schlebusch H, Prietl G, van der Ven H, Krebs D. Serum oxytocin concentration during embryo transfer procedure. *Eur J Obstet Gynecol Reprod Biol* 1999;**1**:77–80.

Drakeley AJ, Jorgensen A, Sklavounos J, Aust T, Gazvani R, Williamson P, Kingsland CR. A randomized controlled clinical trial of 2295 ultrasound-guided embryo transfers. *Hum Reprod* 2008;**23**: 1101–1106.

Harper MJ, Bennett JP, Rowson LE. A possible explanation for the failure of non-surgical transfers in the cow. *Nature* 1961;**190**:789–790.

Hernandez J, Marqueta J, Matorras R, Coroleu B, Simón C, Pérez Milán F *et al.* Registro de inseminaciones (IAC-IAD). Año 2001. Sociedad Española de Fertilidad [AIH-AID registers of the Spanish Society of Infertility]. *Rev Iberoam Fertil* 2006;**22**:91–96.

Knutzen V, Stratton CJ, Sher G, McNamee PI, Huang TT, Soto-Albors C. Mock embryo transfer in early luteal phase, the cycle before in vitro fertilization and embryo transfer: a descriptive study. *Fertil Steril* 1992; **57**:156–162.

Lesny P, Killick SR, Tetlow RL, Robinson J, Maguiness SD. Embryo transfer—can we learn anything new from the observation of junctional zone contractions? *Hum Reprod* 1998;**6**:1540–1546.

Lesny P, Killick SR, Robinson J, Raven G, Maguiness SD. Junctional zone contractions and embryo transfer: is it safe to use a tenaculum? *Hum Reprod* 1999;**9**:2367–2370.

Lewin A, Schenker JG, Avrech O, Shapira S, Safran A, Friedler S. The role of uterine straightening by passive bladder distension before embryo transfer in IVF cycles. *J Assist Reprod Genet* 1997;**1**:32–34.

Mansour RT, Aboulghar MA, Serour GI, Amin YM. Dummy embryo transfer using methylene blue dye. *Hum Reprod* 1994;**7**:1257–1259.

Matorras R, Corcóstegui B, Pérez C, Mandiola M, Mendoza R, Rodríguez-Escudero FJ. Sperm morphology analysis (strict criteria) in male infertility is not a prognostic factor in intrauterine insemination with husband's sperm. *Fertil Steril* 1995;**63**:608–611.

Matorras R, Pérez C, Corcóstegui B, Pijoan JI, Ramón O, Delgado P, Rodríguez-Escudero FJ. Treatment of the male with follicle-stimulating hormone in intrauterine insemination with husband's spermatozoa: a randomized study. *Hum Reprod* 1997;**12**:24–28.

Matorras R, Recio V, Corcostegui B, Rodríguez-Escudero FJ. Recombinant human follicle stimulating hormone versus highly purified urinary FSH: a randomized study in intrauterine insemination with husband's sperm. *Hum Reprod* 2000;**15**:1231–1234.

Matorras R, Urquijo E, Mendoza R, Corcostegui B, Expósito A, Rodríguez-Escudero FJ. Ultrasound-guided embryo transfer improves pregnancy rates and increases the frequency of easy transfers. *Hum Reprod* 2002;**7**:1762–1766.

Matorras R, Corcostegui B, Mendoza R, Ramón O, Aparicio V, Uriarte MT *et al.* Converting an IVF cycle to IUI in low responders with at least 2 follicles. *J Reprod Med* 2003;**48**:789–791.

Matorras R, Mendoza R, Expósito A, Rodriguez-Escudero FJ. Influence of the time interval between embryo catheter loading and discharging on the success of IVF. *Hum Reprod* 2004;**19**:2027–2030.

Matorras R, Ramón O, Expósito A, Corcostegui B, Ocerin I, González-Lopera S *et al.* Gn-RH antagonists in intrauterine insemination: the weekend-free protocol. *J Assist Reprod Genet* 2006; **2**:51–54.

Osuna C, Matorras R, Pijoan JI, Rodríguez-Escudero FJ. One versus two inseminations per cycle in intrauterine insemination with sperm from patients' husbands: a systematic review of the literature. *Fertil Steril* 2004;**82**:17–24.

Sallam HN, Sadek SS. Ultrasound-guided embryo transfer: a meta-analysis of randomized controlled trials. *Fertil Steril* 2003;**4**:1042–1046.

Sallam HN, Agameya AF, Rahman AF, Ezzeldin F, Sallam AN. Ultrasound measurement of the uterocervical angle before embryo transfer: a prospective controlled study. *Hum Reprod* 2002;**7**:1767–1772.

Wainer R, Albert M, Dorion A, Bailly M, Bergére M, Lombroso R, Gombault M, Selva J. Influence of the number of motile spermatozoa inseminated and of their morphology on the success of intrauterine insemination. *Hum Reprod* 2004;**19**:2060–2065.

Woolcott R, Stanger J. Potentially important variables identified by transvaginal ultrasound-guided embryo transfer. *Hum Reprod* 1997; **5**:963–966.

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