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Single-dose depot leuprolide is as efficient as daily short-acting leuprolide in ICSI cycles

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BACKGROUND: In this prospective randomized study, we aimed to compare the efficacy of a single depot (1.88 mg) reduced dose with a daily low dose (0.5 mg/day) of leuprolide for pituitary suppresion as part of controlled ovarian hyperstimulation (COH) in an ICSI program. METHODS: The study population consisted of 103 patients randomized into two groups. Group 1 ($n = 52$) consisted of patients who had daily low-dose leuprolide injections. Group 2 ($n = 51$) consisted of patients who had the 1.88 mg single-dose leuprolide injection. RESULTS: The age of the patients, the number of metaphase II oocytes, the number and quality of embryos transferred were similar between the two groups. Although the length of gonadotrophin stimulation was significantly longer in group 2 ($P < 0.01$), the amount of gonadotrophins used was similar ($P = 0.34$). Leuprolide levels were significantly lower in group 2 within the first 8 h after injection ($P < 0.05$), but no difference was observed thereafter. Although LH levels on the day of hCG ($P = 0.06$) administration and estradiol levels on day 3 ($P < 0.01$) were lower in group 2, LH levels and progesterone levels 1 week after embryo transfer did not show any statistically significant difference. Clinical pregnancy rates per embryo transfer, implantation rates and first trimester abortion rates were also similar for both groups. CONCLUSIONS: A single reduced depot dose (1.88 mg) of leuprolide was found to be as effective as classical long multi-dose protocol for pituitary desensitization in COH for ICSI cycles.

Keywords: GnRHa; leuprolide; ICSI; long protocol

Introduction

The benefits of using GnRHa during controlled ovarian hyperstimulation (COH) in assisted reproductive treatment (ART) cycles is well documented, such as prevention of a premature LH surge, luteinization, a lower cycle cancellation rate (Caspi *et al.*, 1989) and the recovery of a larger number of oocytes (Liu *et al.*, 1992). Among the various types of GnRHa protocols, the long protocol is the one that produces the best clinical pregnancy rates (Daya, 1999). There are two modes of GnRHa administration that can be used to lead to pituitary desensitization in the long protocol, the multi-low-dose or the single depot.

There are a number of studies in the literature comparing the effect of long-acting GnRHa depot preparations with daily short-acting low-dose preparations during COH. Most show no difference in ART outcomes. Single-depot injections for desensitization are more readily accepted by patients, because of its convenience and reduced stress as associated with the multi-low-dose injections (Oyesanya *et al.*, 1995). The clinical concern with the single depot application is the severity of desensitization produced in most patients, which may significantly influence ovarian stimulation and affect pregnancy and miscarriage rates (Devreker *et al.*, 1996; Westergaard *et al.*, 2000). More severe desentization (depressed LH levels) are often associated with longer stimulation periods and greater amounts of gonadotrophins. Much of the debate over the effect of excessive LH suppression is based on studies that are largely retrospective. Although some studies advocate the insignificance of LH during folliculogenesis and claim that low concentrations of circulating LH are sufficient to support development and maturation of follicles and oocytes in normogonadotrophic women (Chappel and Howles 1991; Loumaye *et al.*, 1997) stimulation using gonadotrophins containing a low LH content has been found to result in a lower yield of oocytes when LH concentrations are suppressed below 0.5 IU/l (Fleming *et al.*, 1998).

The minimum dose required for single-depot down-regulation has been investigated, with studies showing that a half-dose injection (1.88 mg), as compared to a full-dose (3.75 mg), seems to be equally effective in pituitary desensitization, with similar duration times for both desensitization and recovery (Balasch *et al.*, 1992). Half-dose injection of depot triptorelin has been successfully used in ovarian stimulation with highly purified FSH during IVF cycles and produce a

higher number of good-quality embryos with a good chance of implantation compared with full-dose preparation (Dal Prato *et al.*, 2004).

However, many clinicians still remain sceptical as regards the use of a half-dose depot for down regulation, because of the few well-structured studies able to unambiguously show its endocrine, embryology and pregnancy benefits. The aim of this study was to determine whether single low-dose (1.88 mg) long-acting leuprolide acetate (LA) administration was effective with regard to pituitary suppression, serum leuprolide levels, hormone levels and clinical outcome variables.

Materials and Methods

The study was conducted in a prospective randomized manner and approved by the ethics committee of Antalya IVF. A signed informed consent was obtained from all patients participating in the study.

Study population

During the study period, 153 women were assessed for eligibility to enroll into the study. Women with decreased ovarian reserve (with <6 antral follicles on baseline ultrasound on day 3 of an unstimulated cycle) were not included in the study as the microdoseflare protocol was the treatment choice for these at our clinic. The eligible study population consisted of 103 patients who underwent 103 ICSI cycles at Antalya IVF. A staff nurse randomized the patients at initiation of stimulation using a computer-generated list. All patients from both groups were instructed to take oral contraceptive pills (OCPs) (Ginera® Schering, Germany) once daily for 21 days. Group 1 ($n = 52$) patients were instructed to administer 0.5 mg/day of LA by subcutaneous (SC) injections (Lucrin daily® Abbott, Turkey) starting from day 16 of OCP. On day 3 of the following cycle, the dosage was reduced to 0.25 mg/day and continued until the day of human chorionic gonadotrophin (hCG) administration. Group 2 ($n = 51$) patients were instructed to administer a single 1.88 mg dose of LA (Lucrin depot® Abbott, Turkey) injection on day 16 of OCP.

COH and oocyte retrieval

After the confirmation of pituitary down-regulation on day 3 of the cycle by sonographic detection of a linear endometrium and suppressed ovaries (no antral follicles >10 mm) and serum estradiol levels of <50 pg/ml, gonadotrophin stimulation with recombinant FSH (Gonal F®, Serono, Turkey) was commenced. The initial FSH dose was 150 IU/day for high responders, 225–300 IU/day for intermediate responders and 450 IU/day for low responders. The initial dose for ovarian stimulation was based upon ovarian reserve indicators, which included the number of antral follicles on day 3 of a previous, basal spontaneous cycle (Chang *et al.*, 1998). The dose was adjusted on day 6 if needed and thereafter in accordance with the patient's response as indicated by her estradiol level and number of developing follicles. Gonadotrophin administration continued until at least two follicles >17 mm in diameter were detected when 10 000 IU hCG was administered followed 35 h later by transvaginal ultrasound-guided oocyte retrieval.

ICSI procedure, embryo transfer and pregnancy assessment

ICSI procedures were performed according to established methods (Tesarik and Sousa, 1995) immediately after oocyte retrieval. Embryo transfers were done under ultrasound guidance with a full bladder using a Wallace catheter (SMS Portex Ltd, UK) ~50 h after oocyte retrieval. Embryos were graded on a scale of 1–4, 1 being the best, according to the criteria described by Balaban *et al.* (2001). Progesterone vaginal gel 90 mg/day was administered for luteal phase support until the day of β-hCG assay. Blood assays for β-hCG measurement were performed 12 days after embryo transfer. Clinical pregnancy was confirmed with the detection of fetal cardiac activity by ultrasound 4 weeks after the embryo transfer.

Serum LH, estradiol, progesterone and leuprolide determination

Blood samples were obtained on day 3 of menses just before gonadotrophin was commenced, on the day of hCG administration and 1 week after embryo transfer for LH activity. Samples were centrifuged at 1000 *g* for 15 min, and sera were frozen and stored at -20°C. LH concentrations were measured by electrochemiluminescence immunoassay (ECLIA) (ECLIA®, Roche, USA). The assay's intra- and inter-assay coefficients of variation were <10 and $<8\%$, respectively, and cross-reaction with hCG was $<0.001\%$.

Blood samples were obtained on day 3 and on the day of hCG administration for the measurement of serum estradiol activity and compared between the groups. Estradiol concentrations were measured by VIDAS 12 device (bioMerieux® Vitek, USA), and the assay's intra- and inter-assay coefficients of variation were <7.5 and $<9.5\%$ respectively.

Serum progesterone levels were measured in blood samples obtained on the day of hCG administration and 1 week after embryo transfer using the Roche Elecsys 1010 immunoassay system (Roche Diagnostics GmbH, Mannheim, Germany). The ECLIA method's intra- and inter-assay coefficients of variation were 2.3 and 4.6%, respectively.

For leuprolide measurements, blood samples were obtained at 0, 2, 4, 8, 24 and 48 h after the depot injection and the first daily injection. Serum leuprolide concentrations were measured with a commercially available radioimmunoassay kit (Bachem, Peninsula Laboratories, Inc., San Carlos, CA, USA) which was specific for leuprolide and did not react with GnRH (0%).

Statistical analysis

LH levels and leuprolide levels were determined as primary outcome variables. The secondary outcomes were: (i) length of gonadotrophin stimulation, (ii) amount of gonadotrophins used, (iii) peak endometrial thickness on day of hCG administration, (iv) number of oocytes retrieved, (v) clinical pregnancy rates per embryo transfer, (vi) implantation rates and (vii) first trimester abortion rates.

Student's *t*-test, Chi-square test and Mann–Whitney *U*-test were used as appropriate. Alpha was considered significant when <0.05.

Results

Recruitment and the follow-up of the patients were done between January and December 2005. All primary demographic factors recorded within each group were similar for both groups (Table 1). While the length of gonadotrophin stimulation was significantly longer in group 2 ($P < 0.01$), the amount of gonadotrophins used was similar for both groups. Distribution of different stimulation doses was also similar between the two groups. The number of oocytes retrieved and number of metaphase II oocytes obtained and number and quality of embryos transferred were not significantly different (Table 2). Clinical pregnancy rates per embryo transfer, implantation rates and first trimester abortion rates were also similar for both groups (Table 2).

Table 1: Basic characteristics and stimulation variables of the two groups

	Group 1[a]	Group 2[a]	*P*-value
Age, years	28.9 ± 4.1	28.5 ± 4.6	0.66*
Age of husband, years	32.8 ± 5.7	32.7 ± 4.5	0.87*
Body mass index	25.5 ± 4.6	24.2 ± 3.1	0.11*
History of IVF failure	6/52 (11.5%)	11/51 (21.5%)	0.17**
Length of stimulation, days	8.6 ± 1.4	9.8 ± 1.4	<0.01*
Gonadotrophin units, IU	3448.7 ± 1733.4	3925.9 ± 3132.9	0.34*
Time between GnRHa and trigger hCG, h	19.9 ± 1.9	20.8 ± 2.5	0.07*
High responders[b]	12 (23%)	15 (29%)	0.46**
Intermadiate responders[b]	15 (29%)	17 (33%)	0.62**
Low responders[b]	25 (48%)	19 (37%)	0.27**
Peak endometrial thickness, mm	10.3 ± 1.7	10.9 ± 2.3	0.08*
Tubal factor	5 (9.6%)	3 (5.8%)	0.71***
Male factor	21 (40.3%)	22 (43.1%)	0.77**
Endometriosis	2 (3.8%)	1 (1.9%)	1.0***
Unexplained infertility	2 (3.8%)	4 (7.8%)	0.43***
Other reasons	22 (42.3%)	21 (41.2%)	0.9**

[a]For description of the groups, see Materials and Methods; [b]For description of the subgroups according to gonadotrophin dose, see Materials and Methods; *Student's *t*-test; **Chi-square test; ***Fisher's exact test.

Although LH levels on day of hCG ($P = 0.06$) and estradiol levels on day 3 ($P < 0.01$) were lower in group 2, LH levels and progesterone levels 1 week after embryo transfer did not show any statistically significant difference (Table 3). Leuprolide levels were significantly lower in group 2 within the first 8 h after injection ($P < 0.05$) (Table 4). We did not encounter severe ovarian hyperstimulation syndrome or any other adverse effects in any group.

Discussion

It has been proposed that a single dose of leuprolide depot may be an equally effective alternative to multiple doses of Buserelin or Nafarelin for pituitary desensitization in women undergoing COH for IVF (El-Nemr *et al.*, 2002). The convenience factor for patients and the simplicity of the management of treatment by clinicians make single dose administration of long-acting GnRH agonists attractive for use in ART treatment cycles. However, the currently available products may achieve severe pituitary desensitization for a protracted period of time, which may necessitate a higher number of gonadotrophin ampules (Ben-Rafael *et al.*, 1991).

In a recent Cochrane review, six studies with a total of 552 women compared the efficacy of long-acting GnRHa with short-acting forms (Albuquerque *et al.*, 2002). The review was not able to show a statistically significant difference between the use of depot GnRHa or daily GnRHa and clinical pregnancy rates per woman. However, the use of depot GnRHa was found to be associated with increased requirements for gonadotrophins and a longer time was needed for ovarian stimulation, which leads to increased costs for IVF treatment (Albuquerque *et al.*, 2002). Only one of the six studies compared low-dose-depot (1.88 mg) leuprolide with short-acting

Table 3. Serum LH, estradiol and progesterone levels of the groups

	Group 1[a]	Group 2[a]	*P*-value
LH_1 on day 3 (mIU/ml)	0.96 ± 0.74	1.03 ± 0.57	0.61*
LH_2 on day of hCG (mIU/ml)	0.86 ± 0.71	0.54 ± 0.47	0.06**
LH_3 1 week after ET (mIU/ml)	0.26 ± 0.37	0.15 ± 0.17	0.79**
E_2 on day 3 (pg/ml)	30.26 ± 30.57	18.50 ± 10.53	<0.01**
E_2 on day of hCG (pg/ml)	3591 ± 2504	4397 ± 2688	0.16*
Progesterone on day of hCG (ng/ml)	2.13 ± 0.96	1.66 ± 0.57	0.11
Progesterone 1 week after ET (ng/ml)	294.9 ± 1162	1018 ± 2256	0.11**

[a]For description of the groups, see Materials and Methods; *Student'S *t*-test; **Mann–Whitney *U*-test.

Table 2: Laboratory and clinical variables

	Group 1[a]	Group 2[a]	*P*-value
Oocyte number	19.2 ± 10.9	22.2 ± 9.8	0.14*
MII oocyte number	13.9 ± 8.9	16.5 ± 8.8	0.14*
Number ET total	4.2 ± 1.2	3.9 ± 1.4	0.24*
Number of ET G1	2.8 ± 1.3	2.9 ± 1.2	0.51*
Number of ET G2	1.2 ± 1.4	0.8 ± 1.2	0.15*
Implantation rate	15.1%	14.8%	0.91**
Clinical pregnancy/ET	17/47 (36.2%)	17/47 (36.2%)	1.0**
First trimester abortion	2/17 (11.7%)	3/17(17.6%)	0.62**

[a]For description of the groups, see Materials and Methods; *Student's *t*-test; **Chi-square test.

Table 4: Serum leuprolide levels (μg/l) of the two groups 2, 4, 8, 24 and 48 h after the initial leuprolide injection

	Group 1[a]	Group 2[a]	*P*-value
After 2 h	29.7 ± 9.0	19.1 ± 5.4	<0.01*
After 4 h	26.9 ± 19.9	15.8 ± 3.0	<0.05**
After 8 h	20.3 ± 20.1	8.6 ± 1.9	<0.05**
After 24 h	25.5 ± 20.2	4.0 ± 1.2	0.08**
After 48 h	19.6 ± 20.5	2.5 ± 1.9	0.09**

[a]For description of the groups, see Materials and Methods; *Student's *t*-test; **Mann–Whitney *U*-test.

multi-dose form (Tsai *et al.*, 1995). Another study incorporated in the review reported a co-intervention with ICSI (Dal Prato *et al.*, 2001).

The strong pituitary suppression and associated higher stimulation costs of full-dose depot GnRHa have been the primary concerns in the initiatives to find a lower dose with sufficient suppresion potential. In this regard, a number of studies have shown that a half dose (1.88 mg) of Triptorelin injection was sufficient to prevent LH surges (Balasch *et al.*, 1992; Dal Prato *et al.*, 2004). Yim *et al.* (2001), comparing full-dose triptorelin with half dose in COH with hMG, found similar IVF outcomes. In another study, highly purified FSH was used, and better implantation and pregnancy rates were reported in half-dose group (Dal Prato *et al.*, 2004). Hsieh *et al.* (2000) examined the effect of a lower dose depot GnRH analogue and a short-acting GnRH analogue in IVF cycles. They found a comparable result in the pregnancy rates of the IVF cycles. However, their study was retrospective, non-randomized and the degree of pituitary suppression and the after-effects were not studied.

Dal Prato *et al.* (2004) reported lower pituitary suppression with half-dose injection reflected by LH levels and claimed that using lower doses of GnRHa may reduce the LH requirement in patients undergoing ART. Lower dose GnRHa may therefore reduce the possible negative effect of GnRHa on ovarian steroidogenesis (Zanagnolo *et al.*, 1996; Parinaud *et al.*, 1988). In our study, LH and progesterone levels on day of hCG administration provided no evidence of premature luteinization in any of our patients in either groups, confirming the effectiveness of half-dose long-acting leuprolide in preventing premature LH surge. It has, however, been reported in the literature that the duration of LH surge is shorter in stimulated cycles compared with the natural cycles (Messinis *et al.*, 1985; Messinis *et al.*, 1986). A single LH assay on the day of hcG, as in our study, may not therefore preclude an LH surge with certainty. The progesterone levels obtained on the day of hCG in our study did, however, help to confirm the lack of premature luteinization.

In our study, group 2 patients had lower serum estradiol levels on day 3, indicating the profound suppression effect of even a half-dose depot preparation. Similar findings regarding estradiol levels were reported with even smaller one-third doses of depot injection (Allahbadia *et al.*, 2004). In the present study, although the length of gonadotrophin stimulation was significantly longer in group 2, the amount of gonadotrophins used was similar which means that the strong pituitary suppression does not increase the amount of gonadotrophin needed. Actually, the use of half-dose depot preparations constituted a real reduction of the total cost of COH. Added to this are the process advantages to both the patient and the clinician, decreased risk of the patient forgetting to administer the drug and, most importantly, it does not interfere in the patient's quality of life, since it substitutes for more than 25 low-dose injections (Geber *et al.*, 2002). Our finding on gonadotrophin use confirms the advantage of using a single low-dose depot; as in other studies, it does not result in a significant increase in the amount of gonadotrophins needed (Tsai *et al.*, 1995; Hsieh *et al.*, 2000). This is in contrast with the findings of Albuquerque *et al.* (2002) who found the amount of gonadotrophins to be significantly higher for the full-dose depot group in their review.

Serum leuprolide levels were lower in group 2 within the first few hours after GnRHa administration (Table 4), which was consistent with previous dose-finding studies (Periti *et al.*, 2002). In their study Periti *et al.* found mean peak plasma leuprorelin concentrations of 13.1 μg/l within 1–3 h of depot SC administration of 3.75 mg, compared with 32–35 μg/l at 36–60 min after a SC injection of 1 mg of a non-depot formulation. Sustained drug release from the depot form maintains plasma concentrations between 0.4 and 1.4 μg/l over 28 days after single 3.75 depot injection. In the present study, some of the patients in group 1 performed daily lucrin injections themselves at home while others preferred to come to the clinic everyday for lucrin administration. Thus, some blood samples for leuprolide at 24 h might have been taken after the lucrin injection. This may have increased the variability of concentrations of leuprolide at the 24 h sample in group 1.

It has been suggested that periovulatory levels of progesterone vary according to ovarian response (Doldi *et al.*, 2005) and elevated progesterone levels >1 ng/ml on hCG administration day, and long protocol agonist cycles seem to adversely affect clinical outcome (Shechter *et al.*, 1994; Ozcakir *et al.*, 2004). In the present study, the progesterone levels 1 week after embryo transfer were similar between the two groups. Our data show that half-dose long-acting LA is also effective in this regard, indicating that it had no detrimental effects on the luteal phase.

Previous work has shown that the continuation of GnRHa administration during the luteal phase does not have any adverse effects on implantation (Fujii *et al.*, 2001).

This is the first study in the literature revealing both the leuprolide levels and outcome variables while comparing half-dose depot leuprolide with low multi-dose leuprolide for pituitary desensitization. Regarding the hormone profile, ICSI outcome and early miscarriages, single-dose 1.88 mg long-acting leuprolide injection is as effective and efficient as classical long multi-dose protocol for GnRHa administration in ICSI cycles. Patient comfort and financial advantage are the two factors that make the low-dose LA depot injection (1.88 mg) a better option than short-acting GnRHa for pituitary suppression in ovarian stimulation for ART. Whether a further reduced dose of GnRHa (e.g. a third or a quarter dose of LA depot) still results in a similar outcome remains to be determined.

References

Albuquerque LE, Saconato H, Maciel MC. Depot versus daily administration of gonadotrophin releasing hormone agonist protocols for pituitary desensitization in assisted reproduction cycles. *Cochrane Database Syst Rev* 2002;**3**:CD002808.

Allahbadia GN, Kadam K, Gandhi G *et al.* The impact of low dose depot Leuprolide Acetate administration in ART patients—a multicentric study. *60th Annual Meeting of the American Society for Reproductive Medicine (ASRM)*. 16–20 mOctober, 2004, (oral abstract presentation).

Balaban B, Urman B, Isiklar A *et al.* The effect of pronuclear morphology on embryo quality parameters and blastocyst transfer outcome. *Hum Reprod* 2001;**16**:2357–2361.

Balasch J, Gomez F, Casamitjana R *et al.* Pituitary-ovarian suppression by the standard and half-doses of D-Trp-6-luteinizing hormone-releasing hormone depot. *Hum Reprod* 1992;**7**:1230–1234.

Ben-Rafael Z, Lipitz S, Bider D *et al.* Ovarian hyporesponsiveness in combined gonadotrophin-releasing hormone agonist and menotropin therapy is associated with low serum follicle-stimulating hormone levels. *Fertil Steril* 1991;**55**:272–275.

Caspi E, Ron-El R, Golan A *et al.* Results of in vitro fertilization and embryo transfer by combined long-acting gonadotrophin-releasing hormone analog D-Trp-6-luteinizing hormone-releasing hormone and gonadotrophins. *Fertil Steril* 1989;**51**:95–99.

Chang M-Y, Chiang C-H, Hsieh T-T *et al.* Use of the antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil Steril* 1998;**69**:505–510.

Chappel S, Howles C. Reevaluation of the roles of luteinizing hormone and follicle stimulating hormone in the ovulatory process. *Hum Reprod* 1991;**6**:1206–1212.

Dal Prato L, Borini A, Trevisi MR *et al.* Effect of reduced dose of triptorelin at the start of ovarian stimulation on the outcome of IVF: a randomized study. *Hum Reprod* 2001;**16**:1409–1414.

Dal Prato L, Borini A, Coticchio G *et al.* Half-dose depot triptorelin in pituitary suppression for multiple ovarian stimulation in assisted reproduction technology: a randomized study. *Hum Reprod* 2004;**19**:2200–2205.

Daya S. Long versus short gonadotrophin releasing hormone agonist protocols for pituitary desensitization in assisted reproduction cycles. The Cochrane Library, issue 3, Update Software, Oxford, 1999.

Devreker F, Govaerts I, Bertrand E *et al.* The long-acting gonadotrophin-releasing hormone analogs impaired the implantation rate. *Fertil Steril* 1996;**65**:122–126.

Doldi N, Persico P, De Santis L *et al.* Serum progesteroni at human chorionic gonadotrohin injection significantly correlate with femali age. *Minerva Ginecol* 2005;**57**:477–482.

El-Nemr A, Bhide M, Khalifa Y *et al.* Clinical evaluation of three different gonadotrophin-releasing hormone analogues in an IVF programme: a prospective study. *Eur J Obstet Gynecol Reprod Biol* 2002;**103**:140–145.

Fleming R, Lloyd F, Herbert M *et al.* Effects of profound suppression of luteinizing hormone during ovarian stimulation on follicular activity, oocyte and embryo function in cycles stimulated with purified follicle stimulating hormone. *Hum Reprod* 1998;**13**:1788–1792.

Fujii S, Sato S, Fukui A *et al.* Continuous administration of gonadotrophin-releasing hormone agonist during the luteal phase in IVF. *Hum Reprod* 2001;**16**:1671–1675.

Geber S, Sales L, Sampaio MA. Comparison between a single dose of goserelin (depot) and multiple daily doses of leuprolide acetate for pituitary suppression in IVF treatment: a clinical endocrinological study of the ovarian response. *J Assist Reprod Genet* 2002;**19**:313–318.

Hsieh Y, Tsai H, Chang C *et al.* Comparison of a single half-dose, long-acting form of gonadotrophin-releasing hormone analog (GnRH-a) and a short-acting form of GnRH-a for pituitary suppression in a controlled ovarian hyperstimulation program. *Fertil Steril* 2000;**73**:817–820.

Liu H, Lai Y, Davis O *et al.* Improved pregnancy outcome with gonadotrophin releasing hormone agonist stimulation is due to the improvement in oocyte quantity rather than quality. *J Assist Reprod Genet* 1992;**9**:338–342.

Loumaye E, Engrand P, Howles C *et al.* Assessment of the role of serum luteinizing hormone and estradiol response to follicle-stimulating hormone on in vitro fertilization treatment outcome. *Fertil Steril* 1997;**67**:889–899.

Messinis IE, Templeton A, Baird DT. Endogenous luteinizing hormone surge during superovulation induction with sequential use of clomiphene citrate and pulsatile human menopausal gonadotrophin. *J Clin Endocrinol Metab* 1985;**61**:1076–1080.

Messinis IE, Templeton A, Baird DT. Endogenous luteinizing hormone surge in women during induction of multiple follicular development with pulsatile follicle stimulating hormone. *Clin Endocrinol* 1986;**24**:193–201.

Oyesanya OA, Teo SK, Quah E *et al.* Pituitary down-regulation prior to in-vitro fertilization and embryo transfer: a comparison between a single dose of Zoladex depot and multiple daily doses of Suprefact. *Hum Reprod* 1995;**10**:1042–1044.

Ozcakir HT, Levi R, Tavmergen E. Premature luteinization defined as progesterone levels >1 on hCG administration day seems to adversely affect clinical Otcome in long gonadotrophin releasing hormone agonist cycles. *J Obstet Gynaecol Res* 2004;**30**:100–104.

Parinaud J, Beaur A, Bourreau E *et al.* Effect of a luteinizing hormone-releasing hormone agonist (Buserelin) on steroidogenesis of cultured human preovulatory granulosa cells. *Fertil Steril* 1998;**50**:597–602.

Periti P, Mazzei T, Mini E. Clinical pharmacokinetics of depot leuprorelin. *Clin Pharmacokinet* 1994;**41**:485–504.

Shechter A, Lunenfeld A, Potashnik G *et al.* The significance of serum progesterone levels on the day of hCG administration on IVF pregnancy rates. *Gynecol Endocrinol* 1994;**8**:89–94.

Tesarik J, Sousa M. Key elements of a highly efficient intracytoplasmic sperm injection technique: Ca2 + fluxes and oocyte cytoplasmic dislocation. *Fertil Steril* 1995;**64**:770–776.

Tsai HD, Chen CM, Lo HY *et al.* Subcutaneous low dose leuprolide acetate depot versus leuprolide acetate for women undergoing ovarian stimulation for in-vitro fertilization. *Hum Reprod* 1995;**10**:2909–2912.

Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. *Hum Reprod* 2000;**15**:1003–1008.

Yim SF, Lok IH, Cheung LP *et al.* Dose-finding study for the use of long-acting gonadotrophin-releasing hormone analogues prior to ovarian stimulation for IVF. *Hum Reprod* 2001;**16**:492–494.

Zanagnolo V, Dharmarajan AM, Hesla J *et al.* Effects of a gonadotrophin-releasing hormone analog on rabbit ovarian function. *Endocrinology* 1996;**137**:5400–5406.

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