JKAU: Med. Sci., **Vol. 14** No. 2, pp: 15-25 (2007 A.D. / 1428 A.H.)

Luteal Supplementation with Estradiol and Progesterone in Patients Stimulated with GnRH Antagonist/rFSH for IVF

Fatma A. Aletebi, FRCS

Department of Obstetrics and Gynecology
Faculty of Medicine
King Abdulaziz University, Jeddah Saudi Arabia
faletebi@kau.edu.sa

*Abstract***.** The use of progesterone for luteal support in stimulated cycles for *in-vitro* fertilization is well established. However, the benefits of the addition of estradiol are still controversial. The aim of this study was to compare ongoing pregnancy rates in patients stimulated with recombinant follicle stimulating hormone and gonadotrophin releasing hormone antagonist for *in vitro* fertilization, who received micronized progesterone for luteal phase supplementation, with or without the addition of estradiol. Thirty-five patients underwent ovarian stimulation with recombinant follicle stimulating hormone and gonadotrophin releasing hormone antagonist and received micronized progesterone for luteal phase supplementation. These patients were compared to 32 other patients who underwent controlled ovarian stimulation with recombinant follicle stimulating hormone and gonadotrophin releasing hormone antagonist and who also received micronized progesterone and estradiol for luteal phase supplementation. There was no significant difference between the two groups for mean-age, duration of infertility or base line follicle stimulating hormone, number of ampoules of gonadotrophin used, number of mature oocyte retrieved, estradiol concentration on the day of injection of human chronic gonadotrophin, fertilization rate or number of embryo transferred. Ten ongoing

Correspondence & reprint requests to: Dr. Fatma A. Aletebi
P.O. Box 80215, Jeddah, 21589 Saudi Arabia
Accepted for publication: 04 March 2007. Received: 7 October 2006

pregnancies were achieved in the progesterone group (28%) and 9 in the progesterone/estradiol group (25%) (p = 0.613). The addition of estradiol to progesterone in the luteal phase after stimulation with recombinant follicle stimulating hormone and gonadotrophin releasing hormone antagonist does not enhance the probability of pregnancy.

Keywords: Estradiol, Gonadotrophin releasing hormone antagonists, Luteal phase support.

Introduction

Luteal phase support is the term used to describe the administration of medication with the aim to support the process of implantation. The luteal phase is defined as the period from occurrence of ovulation until the establishment of a pregnancy or the resumption of menses two weeks later[1].

Luteal phase inadequacy resulting from ovarian stimulation as a cause of *in vitro* fertilization (IVF) failure was established by Edwards and Steptoe in 1980[2]. It is well accepted that luteal phase supplementation is crucial during the time between the disappearance of exogenous human chronic gonadotrophin (HCG) administered for final oocyte maturation and the rise in endogenous HCG during early implantation[3]. However, the 'ideal' scheme of luteal phase support in stimulated cycles has been a matter of debate since the early days of IVF[1].

The role of progesterone as luteal support in stimulated cycles is well established[4]. However, controversy surrounds the benefits of additional supplementation with estradiol (E_2)[5,6]. In a prospective randomized study, evaluated the advantage of adding E_2 valerate 6 mg orally daily to intravaginal micronized progesterone (600 mg daily) as luteal supplementation in 378 infertile women superovulated with a GnRH agonist (GnRHa) and Human Menopausal Gonadotrophin HMG for IVF. The clinical pregnancy rate was similar whether or not E_2 valerate was added to intravaginal progesterone.

The meta-analysis by Pritts and Atwood (2002)[7] suggested that progesterone in combination with E_2 is the best luteal support in long and short agonist protocols. A beneficial effect of E_2 might be related to the dose in which it is used. Lukaszuk *et al*. (2005)[8], in a prospective randomized study, recently evaluated the effect of different E_2 supplementation doses during the luteal phase of implantation and

pregnancy rates in women undergoing intracytoplasmic sperm injection (ICSI) in agonist cycles (n = 231). All subjects received luteal phase support with natural micronized progesterone (Urogestan, Laboratories Besins-Iscovesco, Paris, France), 600 mg/day vaginally in three divided doses, starting on the day of oocyte pickup. Women were randomly allocated to daily doses of 0, 2 or 6 mg of E_2 during the entire luteal phase. It was shown that the addition of a high dose of E_2 to daily progesterone supplementation significantly improved the probability of pregnancy in women treated with a long GnRH analogue protocol for controlled ovarian stimulation (COS). Similarly, Fahri *et al.* (2000)[9], in a prospective randomized study, evaluated the effect of adding E_2 to progestin supplementation during the luteal phase in 271 pg/dl on the day of HCG administration. All patients received progesterone supplementation at a dosage of 150 mg/day divided between 50 mg i.m. injections and 50 mg vaginal tablets, starting on the day after oocyte retrieval. Patients were randomly chosen to receive 2 mg of E_2 (Estrophem; Novo Nordisk, Bagsvaerd, Denmark), given orally, starting on Day 7 after embryo transfer or without any exogenous E_2 supplementation during the luteal phase.

It was shown that for patients who are treated with the long GnRHa protocol for COS, the addition of E_2 to the progestin support regimen has a beneficial effect on pregnancy and implantation rates. However, such an effect could not be shown in the short GnRHa protocol.

Currently, not enough data exists concerning the effect of adding E_2 with progesterone in GnRH antagonist cycles for luteal support. The aim of this study was to compare ongoing pregnancy rates between two different modes of luteal supplementation, using progesterone with and without the addition of E_2 in patients stimulated with recombinant follicle stimulating hormone (rFSH) and GnRH antagonists.

Materials and Methods

Between October 2005 and October 2006, 67 infertile patients underwent *in vitro* fertilization (IVF) cycles and ovarian stimulation with rFSH and GnRH antagonist in a private hospital setting. Those patients were prospectively randomized into two groups. Randomization was based on the consecutive number method. 35 infertile patients underwent ovarian stimulation with rFSH and GnRH antagonist and received

micronized progesterone for luteal phase supplementation. These patients were compared to 32 patients who underwent controlled ovarian stimulation with rFSH and GnRH antagonist and received micronized progesterone and estradiol for luteal phase supplementation.

Recombinant FSH (Gonal F®, Serono) was started in the afternoon of Day 2 of the cycle of 150 IU. To inhibit premature luteinizing hormone (LH) surge, daily GnRH antagonist (Cetrorelix® 0.25 mg, Serono) was used from the day when follicles of ≥ 14 mm were present. Final oocyte maturation was achieved by administration of 10,000 IU of HCG (Profasi®, Serono) as soon as ≥3 follicles of ≥ 17 mm were present. Oocyte retrieval was carried out 36h after HCG administration. Previous studies have described ICSI and IVF procedures in detail[10-13]. Infertile patients underwent ovarian stimulation with rFSH and GnRH antagonist.

Embryos were transferred on Day 3 after oocyte retrieval. Either two or three embryos were transferred per patient based on patient and embryo characteristics[10-13].

Luteal phase supplementation with vaginal administration of 400mg natural micronized progesterone (Crinone® 8%, Serono) was applied, starting one day after oocyte retrieval and continued until 7 weeks of gestation if pregnancy was achieved.

The same treatment as in the previous group was applied with the addition of E_2 valerate (Progyloton®, Schering) 2×2mg/day by mouth. This also started one day after oocyte retrieval and continued until 7 weeks of gestation if pregnancy was achieved. Progyloton® 2 mg was administered once in the morning and once in the evening.

Hormonal assessment was performed at initiation of stimulation and on the day of HCG administration. Additional blood samples were taken as necessary between antagonist initiation and HCG administration. The serum HCG test was performed on days 16 and 18 after the administration of HCG. Serum LH, FSH, HCG, E_2 and progesterone were measured with the automated Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany). Intra- and inter-assay coefficients of variation (CVs) were <3% and <4% for LH; <3% and <6% for FSH; <5% and <7% for HCG; <5% and <10% for E_2; and <3% and <5% for progesterone, respectively.

Ultrasound was performed on Day 6 of stimulation and thereafter as necessary to ensure that HCG was injected on the first day that the patient had ≥ 17 mm. For that purpose, a follicular growth of 2 mm per day was assumed to be normal[14].

The main outcome measure was ongoing pregnancy per patient. Ongoing pregnancy was defined as pregnancy developing beyond 12 weeks. Abortion was defined as births prior to 20 weeks gestation. Statistical analysis was carried out using "Students'" *t* test and *x* 2 tests; $p< 0.05$ was considered to be statistically significant.

Results

Thirty-five infertile patients underwent ovarian stimulation with rFSH and GnRH antagonist and received micronized progesterone for luteal phase supplementation. These patients were compared to 32 other patients who underwent controlled ovarian stimulation with rFSH and GnRH antagonist and who received micronized progesterone and estradiol for luteal phase supplementation. There was no significant difference between the two groups for mean-age, duration of infertility or base line FSH, number of ampoules of gonadotrophin used, number of mature oocyte retrieved, estradiol concentration on the day of injection of HCG, fertilization rate or number of embryo transferred (Table 1).

Table 1. Patient and stimulation characteristics in the progesterone and the progesterone/E_2 group.

	Progesterone	**Progesterone/E_2**	**P value**
No. of patients	35	32	
Mean (±SD) age (years)	30. 2 ± 1.1	30.3 ± 0.99	0.59
Mean (±SD) duration of infertility (years)	13.77 ± 1.77	11.7 ± 1.2	0.97
BMI	22.1 ± 2.1	21.9±1.9	0.56
Mean (±SD) FSH concentration on cycle Day 3	7. 59 ± 1.3	7.48 ± 1.2	0.55
Mean (±SD) LH concentration on cycle Day 3	6.33 ± 0.76	6.43 ± 0.86	0.43
Mean (±SD) no. of rFSH	22.86 ± 5.7	23.3 ± 4.07	0.45
Mean (±SD) concentration of estradiol on the day of HMG administration	1750.46 ± 143.36	1821.3 ± 85.81	0.20

Table 1. Contd.

	Progesterone	Progesterone/E_2	P value
Mean (±SD) concentration of progesterone on the day of HMG administration	0.8 ±0.5	0.6 ±0.3	0.61
Mean (±SD) concentration of LH on the day of HCG administration	7.82 ± 0.79	6.97 ± 0.69	0.94
Mean (±SD) no. of mature oocytes retrieved.	7.46 ± 0.51	8.25 ± 0.35	0.91
Fertilization rates (%)	70.5	68.1	0.45
Mean (±SD) no. of embryos transferred	2.37 ± 0.42	2.5 ± 0.67	0.37
Ongoing pregnancy/transfer (%)	28%	25%	0.61

No significant differences were observed between the groups compared.

Similarly, no significant differences were observed regarding hormonal values in the follicular phase on Day 1 and on the day of HCG administration between the two groups compared (Table 2). The number of developing follicles and the endometrial thickness on the day of HCG was also similar.

Table 2. Hormonal measurements on Day 1 and on the day of HCG administration in patients who received progesterone or progesterone/E_2 for luteal phase supplementation.

	Progesterone group day 1	Progesterone/E_2 group day 1	Progesterone group day HCG	Progesterone/E_2 group day HCG
LH IU/l (mean±SD)	4.92 ± 2.46	5.10 ± 2.34	2.05 ± 2.20	2.1 ± 1.92
E_2 pg/ml (mean±SD)	37.39 ± 14.50	39.21 ± 17.75	1999.7 ± 1233.6	1890.45 ± 1101.69
FSH IU/l (mean±SD)	9.04 ± 3.40	936.0 ± 4.10	13.91 ± 4.62	14.72 ± 3.9
Progesterone pg/ml (mean±SD)	0.74 ± 0.45	0.74 ± 0.37	1.34 ± 0.65	1.30 ± 0.69

The implantation rate per embryo transfer was 37.8% in the progesterone group versus 40.4% in the progesterone/E_2 group ($p = 0.521$).

Ten ongoing pregnancies were achieved in the progesterone group (28%) and 9 in the progesterone/E_2 group (25%) ($p = 0.613$). The early pregnancy losses, which included biochemical pregnancies, ectopic pregnancies and first trimester abortions, were also similar in the two groups (23.5% *vs.* 23.1%, respectively).

Discussion

At the present time only one study is known to exist which has evaluated the role of E_2 for luteal support in patients stimulated with rFSH and GnRH antagonist and the study showed that adding E_2 to progestin does not appear to increase the probability of pregnancy[15]. Our study supports the findings of the previous study. However, the number of patients used was small and further studies with a larger number of patients are needed.

The role of E_2 in the follicular phase is well documented. E_2 is indispensable for endometrial priming and is also responsible for the proliferation of surface epithelium, glands, stroma and blood vessels in endometrium. So far, however, the exact role of E_2 in the luteal phase has not been determined[16]. In the luteal phase of an IVF cycle, serum E_2 and progesterone often decrease to low levels if no hormonal support is provided. The luteal decline in sex steroid levels is associated with reduced implantation and pregnancy rates[17]. It was shown that the addition of 2 mg of E_2 to daily progesterone supplementation significantly improved the probability of pregnancy in women treated with a long GnRH analogue protocol for controlled ovarian stimulation[9]. However, Smitz *et al*. (1993)[6] could not confirm such an affect using a higher E_2 dose supplementation (6 mg/day) in agonist cycles.

No dose-finding studies have been performed so far regarding this issue in antagonist cycles. The choice of 4 mg of E_2 supplementation in the current study was arbitrary. Although the addition of E_2 appears not to be beneficial in the current study using a dose of 4 mg, the probability

that a beneficial effect might be present after supplementations with higher E_2 doses can not be excluded.

The main causes of luteal decline in sex steroid levels (luteal phase defect) are most probably related to the initial high concentrations of E_2 in the early luteal phase[18]. E_2 is involved in the regulation of LH secretion[19] and might cause extremely low LH concentrations in the luteal phase as a result of a strong negative feedback mechanism[20]. On the contrary, high progesterone levels, in the absence of E_2 in the luteal phase, fail to suppress plasma gonadotrophins[18,19].

Different regimens of luteal support to maintain adequate levels of sex steroids have been described[21]. However, the current study finding does not support the addition of E_2 to progesterone in GnRH antagonist cycles.

In spite that the number and quality of the embryos transferred were similar for the two groups compared the similarity in pregnancy rates observed might reflect an adequate endometrial preparation in the two groups.

Interestingly, luteal E_2 depletion in the human does not seem to adversely affect the morphological developmental capacity of the endometrium[16]. Moreover, De Ziegler *et al.* (1992)[18] showed that E_2 is not necessary for the endometrial action of secretion and in regards to the morphological appearance of the endometrium in the luteal phase, it cannot be suggested that E_2 conveys a positive endometrial effect[22].

It might be interesting to study the effect of the addition of E_2 in subgroups of patients stimulated for IVF by GnRHa or antagonists with low levels of E_2 during the luteal phase and to study the effect of different E_2 doses on the pregnancy rate.

In conclusion, the addition of E_2 to progesterone in the luteal phase after stimulation with rFSH and GnRH antagonist does not enhance the probability of pregnancy.

References

[1] **Daya S, Gunby J.** Luteal phase support in assisted reproduction cycles. *Cochrane Database Syst Rev* 2004; **3**: CD 004830.

[2] **Edwards RG, Steptoe PC, Purdy JM.** Establishing full-term human pregnancies using cleaving embryos grown *in vitro*. *Br J Obstet Gynaecol* 1980; **87**(9): 737-756.

[3] **Nyboe Andersen A, Popovic-Todorovic B, Schmidt KT, Loft A, Lindhard A, Hojgaard A, Ziebe S, Hald F, Hauge B, Toft B.** Progesterone supplementation during early gestations after IVF or ICSI has no effect on the delivery rates: a randomized controlled trial. *Hum Reprod* 2002; **17**(2): 357-361.

[4] **Maslar IA**. The progestational endometrium. *Semin Reprod Endocrinol* 1988; **6**: 115–128.

[5] **Ludwig M, Diedrick**. Evaluation of an optimal luteal phase support protocol in IVF. *Acta Obstet Gynecol Scand* 2001; **80**(5): 452-466

[6] **Smitz J, Bourgain C, Van Waesberghe L, Camus M, Devroey P, Van Steirteghem AC.** A prospective randomized study on estradiol valerate supplementation in addition to intravaginal micronized progesterone in buserelin and HMG induced superovulation. *Hum Reprod* 1993; **8**(1): 40-45.

[7] **Pritts EA, Atwood AK.** Luteal phase support in infertility treatment: a meta-analysis of the randomized trials. *Hum Reprod* 2002; **17**(9): 2287-2299.

[8] **Lukaszuk K, Liss J, Lukaszuk M, Maj B.** Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization-embryo transfer cycles. *Fertil Steril* 2005; **83**(5): 1372-1376.

[9] **Farhi J, Weissman A, Steinfeld Z, Shorer M, Nahum H, Levran D.** Estradiol supplementation during the luteal phase may improve the pregnancy rate in patients undergoing in vitro fertilization-embryo transfer cycles. *Fertil Steril* 2000; **73**(4): 761-766.

[10] **Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, Wisanto A, Devroey P.** High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 1993; **8**(7): 1061-1066.

[11] **Devroey P, Tjandraprawira K, Mannaerts B, Coelingh Bennink H, Smitz J, Bonduelle M, De Brabanter A, Van Steirteghem AC.** A randomized, assessor-blind, group-comparative efficacy study to compare the effects of Normegon and Metrodin in infertile female patients undergoing *in-vitro* fertilization. *Hum Reprod* 1995; **10**(2): 332-337.

[12] **Devroey P, van Steirteghem A.** A review of ten years experience of ICSI. *Hum Reprod Update* 2004; **10**(1): 19-28.

[13] **van Landuyt L, De Vos A, Joris H, Verheyen G, Devroey P, van Steirteghem A.** Blastocyst formation in in vitro fertilization versus intracytoplasmic sperm injection cycles: influence of the fertilization procedure. *Fertil Steril* 2005; **83**(5): 1397-1403.

[14] **Kolibianakis EM, Albano C, Camus M, Tournaye H, van Steirteghem AC, Devroey P.** Prolongation of the follicular phase in *in vitro* fertilization results in a lower ongoing pregnancy rate in cycles stimulated with recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. *Fertil Steril* 2004; **82**(1): 102-107.

[15] **Fatemi HM, Kolibianakis EM, Camus M, Tournaye H, Donoso P, Papanikolaou E, Devroey P.** Addition of estradiol to progesterone for luteal supplementation in patients stimulated with GnRH antagonist/rFSH for IVF: a randomized controlled trial. *Hum Reprod* 2006; **21**(10): 2628-2632.

[16] **Younis JS, Ezra Y, Sherman Y, Simon A, Schenker JG, Laufer N.** The effect of estradiol depletion during the luteal phase on endometrial development. *Fertil Steril* 1994; **62**(1): 103-107.

[17] **Hutchinson-Williams KA, Lunenfeld B, Diamond MP, Lavy G, Boyers SP, DeCherney AH.** Human chorionic gonadotropin, estradiol, and progesterone profiles in conception and nonconception cycles in an in vitro fertilization program. *Fertil Steril* 1989; **52**(3): 441-445.

[18] **de Ziegler D, Bergeron C, Cornel C, Medalie DA, Massai MR, Milgrom E, Frydman R, Bouchard P.** Effects of luteal estradiol on the secretory transformation of human endometrium and plasma gonadotropins. *J Clin Endocrinol Metab* 1992; **74**(2): 322-331.

[19] **Nippoldt TB, Reame NE, Kelch RP, Marshall JC.** The roles of estradiol and progesterone in decreasing luteinizing hormone pulse frequency in the luteal phase of the menstrual cycle. *J Clin Endocrinol Metab* 1989; **69**(1): 67-76.

[20] **Beckers NG, Macklon NS, Eijkemans MJ, Ludwig M, Felberbaum RE, Diedrich K, Bustion S, Loumaye E, Fauser BC.** Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in *in vitro* fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. *J Clin Endocrinol Metab* 2003; **88**(9): 4186-4192.

[21] **Nosarka S, Kruger T, Siebert I, Grove D.** Luteal phase support in *in vitro* fertilization: meta-analysis of randomized trials. *Gynecol Obstet Invest* 2005; **60**(2): 67-74.

[22] **Bourgain C, Smitz J, Camus M, Erard P, Devroey P, van Steirteghem AC, Kloppel G.** Human endometrial maturation is markedly improved after luteal supplementation of gonadotrophin-releasing hormone analogue/human menopausal gonadotrophin stimulated cycles. *Hum Reprod* 1994; **9**(1): 32-40.

إضافة هرمون الإستروجين إلى البروجستيررون لزيادة احتمالات الحمل في عمليات أطفال الأنابيب

فاطمة علي العتيبي

قسم النساء والولادة كلية الطب جامعة الملك عبدالعزيز

جـــــدة – المملكة العربية السعودية

المستخلص. تم تقييم إضافة هرمون الإســـتروجين إلـــى هرمـــون البروجيسترون لزيادة احتمالات الحمل في عمليات أطفال الأنابيب. وحيث أنه لا توجد دراسات تبين الاستفادة من هذا الهرمون لزيادة احتمالات الحمل، فقد تمت دراسة ٣٢ مريـــضة خـــضعن لعمليـــة أطفال الأنابيب، وتمت الاستعانة بهرمون الإستروجين، بالإضـــافة إلى هرمون البروجستيرون، وقد قورنـــت النتـــائج بدراســـة ٣٥ مريضة خضعن لعملية أطفال الأنابيـــب بـــدون إضـــافة هرمـــون الإستيروجين في الفترة ما بين أكتوبر ٢٠٠٥ إلى أكتوبر ٢٠٠٦م، وقد تبين عدم وجود اختلاف في معدل الحمل في المجموعتين، وأن إضافة هرمون الإستروجين إلـــى البروجـــستيرون لا يزيـــد مـــن احتمالات الحمل في عمليات أطفال الأنابيب.