Luteal Support for IVF/ICSI Cycles with Crinone 8% (90 mg) Twice Daily Results in Higher Pregnancy Rates Than with Intramuscular Progesterone

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Background: The use of progesterone for luteal support has been demonstrated to be beneficial in assisted reproductive cycles, yet the optimal route of progesterone administration has still not been established. This article is a retrospective study in a tertiary reproductive medical unit to compare luteal progesterone supplementation with vaginal gel or intramuscular progesterone.

Methods: A total of 144 *in vitro* fertilization or intracytoplasmic sperm injection cycles were analyzed, 67 cycles using vaginal gel 90 mg twice daily and 77 cycles using intramuscular progesterone 50 mg daily as luteal support.

Results: Both groups had similar mean age, cause of infertility, baseline hormone levels, dosage of recombinant follicle-stimulating hormone, number of retrieved and fertilized oocytes, and number of transferred embryos. The vaginal gel group had significantly lower mid-luteal serum progesterone levels but higher implantation rate (32.5% *vs.* 18.5%, $p = 0.001$) and ongoing pregnancy rate (55.2% *vs.* 32.5%, $p = 0.006$). Within each group, mid-luteal serum progesterone levels between pregnant or non-pregnant patients were comparable. For patients with serum estradiol levels on day of human chorionic gonadotropin greater than 5,000 pg/mL, vaginal gel still resulted in better ongoing pregnancy and implantation rates.

Conclusion: The use of vaginal progesterone gel twice daily for luteal support results in better pregnancy outcomes than intramuscular progesterone. A high local progesterone effect from vaginal gel might improve endometrial receptivity under extraordinarily high serum estradiol levels. [*J Chin Med Assoc* 2008;71(8):386–391]

Key Words: assisted reproductive technology, Crinone, luteal phase, pregnancy rate

Introduction

During assisted reproductive technology (ART) treatment, the use of gonadotropin-releasing hormone (GnRH) agonists and the aspiration of follicular fluid can lead to a relative progesterone deficit and inappropriate preparation of the endometrium for embryo implantation. Supplementation of progesterone or human chorionic gonadotropin (hCG) in the luteal phases after *in vitro* fertilization (IVF) cycles significantly improves fertility outcomes compared with no treatment.[1] No significant difference has been found between hCG and progesterone in terms of pregnancy or miscarriage rates, but the odds of ovarian hyperstimulation syndrome (OHSS) are more than 2-fold higher with treatment involving hCG.[2]

Progesterone can be administered by oral, intramuscular or vaginal routes, but the optimal route has not yet been established. Although there is increasing evidence that vaginal and intramuscular progesterone are at least equally effective in IVF treatment outcomes,[3,4] lower clinical pregnancy and delivery rates when using vaginal progesterone rather than intramuscular progesterone-in-oil has been reported in a meta-analysis of randomized trials.[5] However, through the use of vaginal progesterone, painful application of intramuscular

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injections and their complications, such as local soreness, abscesses, and inflammatory reactions, were avoided.[6]

High serum estradiol levels achieved through ovarian hyperstimulation have been claimed to reduce endometrial receptivity,[7] and progesterone has been found to improve implantation by regulating the immune response.[8] Vaginal gel can produce significantly higher endometrial progesterone levels than intramuscular injection, and vaginal progesterone gel is used twice daily rather than once daily. The purpose of this study was to compare the efficacy of these 2 forms of luteal phase support during IVF and intracytoplasmic sperm injection (ICSI) cycles, with ongoing pregnancy rate as the primary outcome.

Methods

This retrospective study was approved by the Institutional Ethics Review Board of National Taiwan University Hospital. All patients recruited in this study underwent complete infertility evaluation, including early follicular phase serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol levels. Controlled ovarian hyperstimulation was performed using the long GnRH agonist protocol. Buserelin nasal spray (Supremon, Hoechst, Frankfurt am Main, Germany), 200 μg four times daily, was administered 7 days before the estimated start of the next menses. After downregulation was achieved (menstrual bleeding occurred and serum estradiol < 50 pg/mL), the dose of buserelin was halved (100 μg four times daily) and ovarian stimulation was commenced the next day with a daily subcutaneous dose of 200 IU recombinant FSH (Puregon; Organon, Oss, The Netherlands). After giving 4 days of recombinant FSH, transvaginal ultrasonography and serum hormone analysis (estradiol, progesterone, LH) were performed every other day, and the dose of recombinant FSH was adjusted according to the ovarian response.

When at least 2 follicles had reached a diameter of 18 mm or more, buserelin nasal spray and recombinant FSH were stopped and a single bolus of 10,000 IU hCG (Profasi; Serono, Geneva, Switzerland) was administered intramuscularly. Ultrasound-guided transvaginal oocyte retrieval was then performed 34–36 hours after hCG administration. Subsequently, IVF or ICSI was performed. Embryo transfer was done on day 3 after oocyte retrieval. According to the guidelines of the Taiwan Society for Reproductive Medicine, no more than 3 embryos could be transferred to women < 35 years of age, and no more than 4 embryos to women aged ≥ 35 years. Luteal support commenced 2 days after oocyte retrieval.

We analyzed all the fresh IVF or ICSI cycles conducted by 2 attending physicians of National Taiwan University Hospital from September 2005 to April 2007. These 2 doctors had different luteal support protocols: one used oral estradiol valerate (Estrade; Synmosa, Taipei, Taiwan) 6 mg twice daily combined with vaginal progesterone gel (Crinone 8%; Fleet Laboratories, Watford, UK) 90 mg twice daily, and the other used estradiol valerate 6 mg twice daily with intramuscular progesterone-in-oil (Progesterone; Tai Yu, Hsinchu, Taiwan) 50 mg daily. In total, there were 67 IVF or ICSI cycles using Crinone and 77 cycles using intramuscular progesterone in this study.

Mid-luteal serum progesterone levels were obtained 9 days after oocyte retrieval. Serum hCG levels were checked 16 days after oocyte retrieval, and a level above 50 IU/L was considered positive. Ultrasound examination was performed 1 week later to confirm clinical pregnancy and determine the number of intrauterine gestational sacs. The implantation rate was defined as the ratio of the number of gestational sacs to the number of embryos transferred. The presence of at least 1 viable fetus at 12 weeks of gestation was classified as ongoing pregnancy. Serum FSH, LH, estradiol and progesterone levels were measured by means of chemiluminescence immunoassay (Immulite 2000; DPC, Flanders, NJ, USA). Patients' serum samples were assayed immediately upon sample acquisition.

The 2 progesterone supplementation protocols were retrospectively compared for implantation rate, clinical pregnancy rate and ongoing pregnancy rate. Mid-luteal serum progesterone levels were compared between clinically pregnant and non-pregnant patients. Ongoing pregnancy and implantation rates between high (≥ 5,000 pg/mL) and low (< 5,000 pg/mL) serum estradiol levels on the day of hCG administration were also analyzed. All data were analyzed using the commercially available software package SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) and presented as mean ± standard deviation or number (%). Statistical analysis was carried out using Mann-Whitney U test for continuous data and χ^2 test for categorical data. Differences were considered to be significant when $p < 0.05$.

Results

The demographic data and treatment outcomes of the 2 luteal support protocols are shown in Table 1. There were no significant differences between the protocols in terms of age distribution, infertility causes, baseline

Table 1. Demographic data and treatment outcomes of both luteal support protocols*

	Vaginal gel ($n=67$)	Intramuscular ($n=77$)	p
Age (yr)	32.5 ± 4.0	33.5 ± 3.4	0.156
> 35	25 (37)	34 (44)	0.405
> 38	4 (6)	6 (8)	0.668
Cause of infertility			0.399
Tubal factor	10 (15)	20 (26)	
Endometriosis	7 (10)	9 (12)	
Male factor	40 (60)	38 (49)	
Unexplained infertility	10 (15)	10 (13)	
Baseline hormone levels			
FSH (U/L)	6.17 ± 1.97	6.36 ± 2.12	0.702
Luteinizing hormone (U/L)	4.51 ± 2.23	4.76 ± 3.01	0.981
Estradiol (pg/mL)	32.7 ± 17.0	31.5 ± 12.8	0.918
Hormone levels on day of hCG			
Estradiol (pg/mL)	4,380 ± 2,123	4,404 ± 2,056	0.949
Progesterone (ng/mL)	1.10 ± 0.55	1.29 ± 0.69	0.070
Total dose of r-FSH (IU)	1515 ± 443	1671 ± 720	0.114
Retrieved oocytes	21.3 ± 6.3	20.7 ± 7.0	0.503
Fertilized oocytes	14.0 ± 5.4	13.6 ± 5.5	0.403
Transferred embryos	2.90 ± 0.47	2.95 ± 0.48	0.401
Mid-luteal serum progesterone (ng/mL)	13.8 ± 5.9	41.1 ± 56.5	< 0.001
Implantation rate	63/194 (32.5)	42/227 (18.5)	0.001
Clinical pregnancies	38 (56.7)	27 (35.1)	0.009
Ongoing pregnancies	37 (55.2)	25 (32.5)	0.006

**Data presented as mean ± standard deviation or n (%). FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin; r-FSH = recombinant FSH.*

hormone levels, dosage of recombinant FSH, serum estradiol and progesterone levels on the day of hCG administration, number of oocytes retrieved, number of oocytes fertilized, or number of embryos transferred.

Mid-luteal serum progesterone levels were significantly lower (13.8 ± 5.9 *vs.* 41.1 ± 56.5 ng/mL, $p < 0.001$) in the patients using vaginal progesterone gel, but the implantation rate (32.5% *vs.* 18.5%, $p = 0.001$), clinical pregnancy rate (56.7% *vs.* 35.1%, $p = 0.009$) and ongoing pregnancy rate (55.2% *vs.* 32.5%, $p = 0.006$) were all significantly higher in this group. There were 4 triplet and 13 twin pregnancies in the vaginal gel group, and 2 triplet and 7 twin pregnancies in the intramuscular progesterone group. In each protocol, there was 1 patient who suffered from moderate/severe OHSS, and both patients recovered smoothly after inpatient supportive care.

In each protocol, the mid-luteal serum progesterone levels were similar between patients with and without clinical pregnancy (Table 2). For patients with high (≥ 5,000 pg/mL) serum estradiol levels on the day of hCG administration, vaginal progesterone gel still resulted in better ongoing pregnancy and implantation rates (Table 3). There was no significant difference in ongoing pregnancy and implantation rates between patients with high and low serum estradiol levels in the vaginal gel group and in the intramuscular progesterone group.

Table 2. Mid-luteal serum progesterone levels in clinically pregnant and non-pregnant patients

	Pregnant	Non-pregnant	p
Vaginal gel	$n=38$	$n=29$	
Progesterone (ng/mL)	14.4 ± 6.0	13.1 ± 5.9	0.386
Intramuscular	$n=27$	$n=50$	
Progesterone (ng/mL)	36.1 ± 32.6	43.8 ± 66.3	0.580

Discussion

In this retrospective study, we compared 2 protocols of luteal support and found that Crinone 8% (90 mg)

Table 3. Ongoing pregnancy rates and implantation rates with different serum estradiol levels on the day of human chorionic gonadotropin administration

	Serum estradiol ≥ 5,000 pg/mL	Serum estradiol < 5,000 pg/mL	*p*
Ongoing pregnancy rate			
Vaginal gel	14/25 (56%)	23/42 (54.8%)	0.921
Intramuscular	8/28 (28.6%)	17/49 (34.7%)	0.581
p	0.043	0.055	
Implantation rate			
Vaginal gel	24/73 (32.9%)	39/121 (32.2%)	0.926
Intramuscular	11/80 (13.8%)	31/147 (21.1%)	0.174
p	0.005	0.039	

twice daily resulted in higher ongoing pregnancy rates than intramuscular progesterone 50 mg daily.

Normal luteal function is essential for maintaining first-trimester pregnancy, but corpora lutea may be compromised during controlled ovarian hyperstimulation or oocyte retrieval. Luteal support in IVF/ICSI cycles with progesterone or hCG is a standard procedure to improve pregnancy outcomes, but hCG increases the odds of OHSS, a relatively common and potentially life-threatening complication of ovarian stimulation. When using hCG for luteal support, our experience shows that the overall frequency of severe OHSS is 5.5%.[9] Although luteal supplementation with progesterone could not eradicate OHSS, the risk was markedly decreased to 1.4% (2/144) in the present study.

The pharmacokinetic profiles of vaginal and intramuscular progesterone administration are extremely different: intramuscular injection produces high plasma levels and low endometrial levels, but the opposite occurs with vaginal gel application.[10–12] Historically, luteal adequacy was judged by the measurement of serum progesterone concentrations, but we did not find any significant difference in mid-luteal progesterone levels between pregnant and non-pregnant patients.

Despite low serum progesterone levels with vaginal administration, the substantial local effect may induce full secretory transformation of the endometrial stroma.[13] But some studies reported that using vaginal progesterone gel compared with intramuscular progesterone-in-oil resulted in higher rates of biochemical pregnancy loss,[14] lower implantation rates,[15–17] and lower ongoing pregnancy rates.[18] The definite explanation is not clear, but lower serum progesterone levels when using vaginal gel may lead to a higher systemic estradiol-to-progesterone ratio, and decreased implantation efficiency in this setting has been reported.[19]

Most previous studies used Crinone 90 mg vaginal progesterone gel daily, not 90 mg twice daily. The higher frequency of vaginal progesterone gel might compensate for the effect of high systemic estradiol-to-progesterone ratio and also maintain normal endometrial histology. In a study evaluating oocyte donation model,[20] Crinone 90 mg twice daily achieved comparable ongoing pregnancy rates to intramuscular progesterone administration. In that study, all adequate endometrial biopsy specimens from patients using Crinone were in phase.

Extremely high serum estradiol levels achieved through ART cycles have been claimed to cause lower pregnancy rates by having adverse effects on endometrial receptivity.[7,21–23] Extraordinarily high serum estradiol levels (≥ 5,000 pg/mL) are associated with severe downregulation of the expression of endometrial progesterone receptors,[24] asynchronous endometrial development,[25] and suboptimal endometrial perfusion.[26] High serum estradiol concentrations may also reduce endometrial expression of T helper type 2 (Th2) cytokines such as interleukin-11 and interleukin-6,[27] and there is evidence to show that pregnancy rejection is mediated by T helper type 1 (Th1) cytokines, whereas Th2 cytokines play an important role in sustaining pregnancy.[28]

Progesterone has been found to specifically block Th1 immunity to trophoblasts,[8] therefore high local progesterone concentrations from vaginal gel might decrease the detrimental effect of high systemic estradiol levels. We used 5,000 pg/mL as the cut-off value of serum estradiol levels on the day of hCG administration and compared the ongoing pregnancy and implantation rates. At both high and low estradiol levels, the vaginal gel group still had better ongoing pregnancy and implantation rates, but the difference in ongoing pregnancy rates was marginally significant (54.8% *vs.* 34.7%, $p = 0.055$) for patients with low estradiol levels. In the intramuscular progesterone group, although without statistical significance, there were differences of more than 6% in absolute terms in both ongoing

pregnancy rates (28.6% *vs.* 34.7%, $p=0.581$) and implantation rates (13.8% *vs.* 21.1%, $p=0.174$) in favor of low over high serum estradiol levels.

In the mid-luteal phase of IVF–embryo transfer cycles without hormone supplementation, serum estradiol and progesterone often decrease to low levels.[29] Subnormal mid-luteal estradiol concentrations have been associated with endometrial maturation delay and reduced endometrial receptivity.[30] Estrogen supplementation can result in a significant elevation of serum estradiol levels, and the addition of estrogen to progesterone supplementation in the luteal phase can significantly improve pregnancy and implantation rates in patients treated with the long GnRH agonist protocol.[31,32] Accordingly, we gave estrogen as part of luteal phase support for all patients after controlled ovarian hyperstimulation.

In conclusion, adequate tissue level of progesterone can be obtained after the vaginal administration of progesterone gel, and luteal adequacy should not be judged only by serum progesterone concentrations. Compared with intramuscular progesterone-in-oil, Crinone 8%, 90 mg twice daily significantly improves pregnancy outcomes. Vaginal gel might also improve endometrial receptivity under extraordinarily high serum estradiol levels. Although significant limitations of a retrospective analysis exist, the patient-specific characteristics of the 2 groups were generally similar. More prospective randomized trials are needed to find the best route of luteal supplementation in ART treatment.

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