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ORIGINAL ARTICLE

Evaluation of an optimal luteal phase support protocol in IVF

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Subject. Luteal phase support has been shown in the past to be an essential part of ovarian stimulation protocols, especially the long protocol. It could be shown that hCG is as effective as is progesterone for luteal phase support but hCG is accompanied by a higher rate of complications.
Methods. Progesterone can be administered in several routes. The oral, intramuscular (i.m.) and vaginal routes have been chosen frequently in the past. The oral route is ineffective, since progesterone has a low oral bioavailability (<10%), and a high rate of metabolites, which may result in side effects such as somnolence etc. Intramuscular administration provides very high serum levels of progesterone and this route is effective with regard to pregnancy rates. Injection of progesterone, however, is painful and cannot be done by the patient herself. The vaginal route is also effective, progesterone can be administered by the patient herself and progesterone is delivered directly to the uterus, where high levels are achieved (first uterine pass effect).
Results. Several studies could show, in the past, that the vaginal administration of progesterone is effective also with regard to the downregulation of uterine contractions. Crinone® 8% Vaginal Gel is especially designed for vaginal use with a special applicator and has to be administered once daily in the morning. It adheres to the vaginal epithelium, and leakage of the gel is substiantially reduced as compared to other drugs like capsules or suppositories.
Conclusions. Since progesterone is as effective as hCG for luteal phase support but provides a higher safety with regard to ovarian hyperstimulation syndromes, and vaginal progesterone is as effective as intramuscular progesterone, vaginal progesterone should be the standard choice for luteal phase support. Crinone® 8% seems to be the most comfortable way of vaginal administration of progesterone for luteal phase support in IVF cycles.

Key words: human chorionic gonadotrophin; luteal phase support; ovarian hyperstimulation syndrome; progesterone

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In the context of assisted reproduction procedures, luteal phase support is the term used to describe hormone administration during the second phase of the stimulation cycle. The drugs normally used for luteal phase support are progesterones, estrogens and human chorionic gonadotrophin (hCG). Progesterone and estrogen administration is in effect hormone supplementation, whereas hCG is used to stimulate these hormones in the corpus luteum.

The use of hCG is driven by the hypothesis that, in addition to progesterone and estrogen, the corpus luteum produces other hormones which are required for endometrial transformation and optimization of the conditions for embryo implantation

Abbreviations:
CNS: central nervous system; FSH: follicle-stimulating hormone; GABA: γ-aminobutyric acid; GnRH: gonadotrophin-releasing hormone; hCG: human chorionic gonadotrophin; hMG: human menopausal gonadotrophin; IVF: *in vitro* fertilization; LH: luteinizing hormone; OHSS: ovarian hyperstimulation syndrome; RIA: radioimmunoassay.

and development. hCG is, however, a well-known trigger for ovarian hyperstimulation syndrome (OHSS), a potentially life-threatening condition associated with an increased risk of thromboembolism. One of the key goals of luteal phase support is, therefore, to minimize the risk of OHSS.

The question of optimal luteal phase support is raised repeatedly, with many people still favoring the use of hCG, despite the risk of OHSS. This paper presents and discusses current data in the field of luteal phase support, and compares the various options available. The aim is to uncover a protocol which is simple, yet effective, as well as being acceptable to patients.

Numerous protocols exist for ovarian stimulation, though the 'long' protocol is now considered to be the standard protocol and is used in over 70% of *in vitro* fertilization (IVF) cycles in Germany (1). This paper, therefore, focuses on luteal phase support in relation to the use of this form of ovarian stimulation. However, data from relevant studies, which have not specified the ovarian stimulation protocol used, have been included as appropriate.

Theories relating to luteal phase insufficiency following ovarian stimulation

Edwards and Steptoe (2) were the first to suggest that luteal phase support was a requirement for optimal outcome after IVF. Other investigators subsequently showed that ovarian stimulation, as part of a long protocol, did, indeed, lead to an endocrinological disturbance during the luteal phase (3, 4). A meta-analysis of prospective, randomized, studies showed that luteal phase support was clearly beneficial in establishing a pregnancy after IVF, following stimulation as part of a long protocol (5). This observation will be discussed in more detail further on in this article.

Luteal phase insufficiency may be related to one or more of the following effects, each of which can be both supported and discounted by published data:

- supraphysiological estradiol concentrations as a result of multifollicular maturation;
- supraphysiological progesterone concentrations immediately after ovulation as a result of multiple corpora lutea;
- suppression of endogenous luteinizing hormone (LH) secretion during the luteal phase as a result of persistent pituitary suppression through the use of GnRH agonists.

The administration of high doses of estrogen after coitus results in a well-known contraceptive effect, particularly in combination with progestogens (morning-after pill) (6). The precise mechanism of action is uncertain, but may be related to impairment of corpus luteum function, an effect which has been clearly demonstrated in monkeys (7, 8). In humans, investigators have shown that the use of ethinylestradiol can induce a fall in serum progesterone levels and a shortening of the luteal phase (9). It is unclear, however, why especially stimulation cycles using GnRH agonists – which require luteal phase support, as almost all forms of ovarian stimulation for IVF – would result in supraphysiological serum estradiol levels. Additionally, although high doses of synthetic progestogens consistently lead to premature luteolysis, this is not the case with natural progesterone (10, 11).

Studies in humans and other primates, from the early 1970s, show that the corpus luteum requires a consistent LH stimulus, the cumulative value of which has been calculated to be about 400 IU/day, to perform its physiological function. These data are supported from animal experiments in which monkeys, immunized with hCG antiserum with a cross-reaction to LH, exhibited premature luteolysis and a shortened menstrual cycle (12–14).

Although this model may explain why luteal phase insufficiency occurs as a result of persistent pituitary suppression due to depot GnRH agonist administration or to GnRH agonist accumulation following daily administration, it does not explain luteal insufficiency following the use of a GnRH antagonist (15, 16). In the 1998 study by Albano et al. (15), six patients were treated using a GnRH antagonist protocol without luteal phase support. Of the six patients, three had a shortened luteal phase ($\leq$12 days) and none became pregnant (15). It is generally believed that the (supplemented) luteal phase is endocrinologically independent of the dose of GnRH antagonist used (16). This belief correlates with observations that, after daily administration of cetrorelix at doses of either 0.5 mg or 0.25 mg (17), or after a single 3 mg dose in the middle of the luteal phase (18), this agent is barely detectable in plasma on the day of embryo transfer. Therefore, no long-term effect on the pituitary would be anticipated. The administration of hCG for ovulation induction may lead to persistent suppression of the pulsatile activity of the pituitary which, under normal circumstances, is necessary to maintain the corpus luteum.

In summary, the most likely mechanism for luteal phase insufficiency is a disturbance of pituitary function, possibly in conjunction with an elevated serum estradiol concentration following ovarian stimulation as a result of multiple follicular maturation.

Luteal phase insufficiency following ovarian stimulation: results of prospective, randomized studies

In this section, the extent to which luteal phase support is necessary following ovarian stimulation as part of a long protocol is evaluated.

In one early, prospective, study, 115 patients received either hCG ($n=61$) or no hormone support ($n=54$) during the luteal phase (19). The investigators reported a significantly higher pregnancy rate among patients who had received hCG compared with those patients who had not (41.0% *vs* 14.8%, respectively).

Further studies confirmed this result. In a prospective, randomized, multicenter, double-blind, placebo-controlled study, patients were given luteal phase support with either hCG (193 embryo transfers) or placebo (194 embryo transfers) following ovarian stimulation with hMG. A significantly better pregnancy rate was achieved in patients who had received hCG rather than placebo (18.7% *vs* 9.3%, respectively) (20). In the same year, the results of a fairly small, prospective, randomized study were published which compared pregnancy rates following luteal phase support with either hCG (18 embryo transfers) or no supplementation (18 embryo transfers) after ovarian stimulation with hMG (21). The pregnancy rates were 50% (9/18) and 17% (3/18) after hCG and no hormonal supplementation, respectively, suggesting that hCG conferred a clear advantage. However, there was also a statistically significant increase in the rate of OHSS among patients who received hCG ($p<0.03$). There were five cases of OHSS in the hCG group, three at WHO Grade II and two at WHO Grade III, and no cases of OHSS in patients not receiving luteal phase supplementation.

In a more recent, prospective, randomized, placebo-controlled study, Abate et al. (22) showed that the pregnancy rate per embryo transfer over 43 cycles was significantly higher during 17α-hydroxyprogesterone caproate (341 mg, i.m., every 3 days) administration (32.5%) than it was during placebo administration (saline solution, i.m. every 3 days) (18.3%). Progesterone treatment was initiated within 24 hours following embryo transfer and was continued up to the 12th week of pregnancy if a pregnancy occurred, or up to the day on which hCG levels were determined (22).

In a meta-analysis which includes data from the studies by Smith et al. (19) and Herman et al. (21) reported above, it was concluded that luteal phase support during a long protocol is of clinical benefit (5).

hCG administration for luteal phase support in the long protocol

The need for luteal phase support leads to the question that was raised at the beginning of this article – that is, which agents should be used for luteal phase support, in terms of both efficacy and safety? Conventionally, hCG is the drug of choice and will, therefore, be compared with studies which have used progesterone.

Despite the variety of protocols that exist for the use of hCG in luteal phase support, there appears to be no rationale behind either the dose frequency (every 2 days, every 3 days, varying intervals) or the total daily dose (1000, 2500 or 5000 IU).

For example, a prospective, randomized, study conducted in 1988 involved 91 patients, 50 of whom received micronized vaginal progesterone (600 mg/day) in combination with oral estradiol valerate (6 mg/day), and 41 who received hCG (2000 IU on days 4, 8 and 12 of the luteal phase). The pregnancy rates were 32.0% and 31.7%, respectively, which were, therefore, entirely comparable (23).

In another prospective, randomized, study describing 121 cycles where ovarian stimulation was achieved with hMG, luteal phase support was provided in the form of hCG ($n=72$), 3×1500 IU, or progesterone ($n=49$), 25 mg/day, i.m. In all cases, the administration of progesterone was continued after the positive beta hCG test until a heartbeat was detected. Comparable pregnancy rates were found in both groups (18.1% *vs* 17.3% in the hCG- and progesterone-treated patients, respectively) (24).

In a study by Buvat et al. (25), luteal phase support was provided using oral progesterone – a preparation which is considered to be inadequate due to a low oral bioavailability for this purpose, and which was already discussed in their report by the authors. The study compared luteal phase support with either oral, micronized progesterone (400 mg/day) or hCG (3×1500 IU) in 171 embryo transfer cycles. Data analysis was carried out on 140 embryo transfer cycles only (70 transfers in each group), as women with serum estradiol levels which exceeded 2700 pg/ml estradiol on the day of hCG were excluded. The use of hCG led to significantly better implantation (19.0% *vs* 7.5%) and pregnancy rates (31.4% *vs* 14.3%). Due to the low bioavailability described above, these data are excluded from the meta-analysis by Soliman et al. (5), there does not appear to be a difference between the performance of hCG and progesterone for luteal phase support (5).

Two further studies were published after this meta-analysis. In the first, 77 patients were pros-

pectively randomized after ovarian stimulation, and received either 2000 IU hCG (4×) (n=38) or 50 mg progesterone i.m. daily (n=39). Pregnancy rates were similar in the hCG and progesterone groups – 36.7% *vs* 35.3%, respectively. The implantation rate was also comparable between groups: 12% *vs* 14% in the hCG- and progesterone-treated patients, respectively (26). However, the incidence of moderate or severe OHSS was higher in patients who received hCG. The authors, therefore, advised against supplementation with hCG, particularly in women with relatively high serum estradiol levels.

Finally, the efficacy of vaginal progesterone alone (400 mg daily) (n=89) and in combination with hCG (day +3, +6, +9 and +12, 1500 IU on each occasion starting from the day of oocyte retrieval) (n=89) has been studied (27). OHSS developed in 11 out of the 89 cycles supplemented with hCG, therefore, no additional hCG was administered beyond the second hCG injection. There was no separate analysis of these cycles and they were not excluded from the overall analysis. The clinical pregnancy rates were not significantly different between groups – 26% after the administration of progesterone alone and 15% after the combined supplementation (relative risk 0.49; 99% confidence interval 0.18–1.3) (27). The authors also report that serum progesterone and estradiol levels were significantly higher after hCG administration (days 6, 9 and 12 after oocyte retrieval; $p<0.001$), although this did not affect the pregnancy rate.

In another prospective, randomized study, Herman et al. (28) investigated the benefits of a single dose of 2500 IU hCG, administered to patients whose progesterone levels were less than 50 ng/ml and whose mid-luteal estradiol concentrations were low (<1000 pg/ml) (28). A total of 170 IVF cycles were analyzed, comprising patients who had a serum estradiol level of > 2000 pg/ml on the day of hCG. Luteal phase support was provided during all of the cycles in the form of intramuscular progesterone (50 mg/day). Pregnancy rates were comparable between the two groups – 31% in women given hCG *vs* 29% in women not given hCG – suggesting that pregnancy rate was not influenced either by the additional dose of hCG or the significantly lower progesterone levels. Thus, there was no evidence that hormone-dependent hCG administration was beneficial (Table I).

Although one study has evaluated the use of progesterone and estradiol valerate in comparison with hCG for luteal phase support (23), there has not yet been a direct comparison of vaginally administered progesterone and hCG. In 1996, Mochtar et al. (27) performed a study using progesterone for luteal phase support both alone and in combination with hCG. No difference was detected between the two regimens, though the dose of progesterone (400 mg/day) used during the study was somewhat lower than the internationally accepted standard of 600 mg/day. The dose of hCG was tailored according to the risk of OHSS, though this was not taken into consideration in the final analysis (27).

Our group, therefore, conducted a prospective, randomized study involving 413 patients, stratified into two OHSS risk groups (64). In the group which was at high risk of developing OHSS (estradiol ≥2500 pg/ml on the day of hCG and/or ≥12 oocytes at oocyte retrieval), patients were randomized to receive either progesterone alone or in combination with a single 5000 IU dose of hCG on the day of embryo transfer. The second group comprised patients at a low risk of developing OHSS (estradiol <2500 pg/ml and <12 oocytes). Patients in this group received one of three possible treatment options:

- vaginal progesterone (Utrogestan®); two capsules administered three times daily (equivalent to 600 mg/day) beginning on the evening prior to embryo transfer;
- hCG (5000 IU) on the day of embryo transfer, 5000 IU 3 days later and 2500 IU 6 days later;

Table I. Outcome after mid-luteal administration of 2500 IU hCG to women with low estradiol levels (<1000 pg/ml) who were receiving luteal phase support with intramuscular progesterone (50 mg). Values shown are means±standard deviation unless otherwise stated (28)

	Group		
	2500 IU hCG (n=85)	No hCG (n=85)	Significance value
Age (years)	31.2±0.4	32.2±0.6	n.s.
Estradiol concentration on day of hCG administration (pg/ml)	2586±80	2473±79	n.s.
Number of oocytes	17.4±0.8	18.3±0.7	n.s.
Number of embryos/transfer	2.8±0.1	2.6±0.1	n.s.
Implantation rate (%)	35/252 (13.9)	32/234 (13.7)	n.s.
Number of pregnancies (%)	26 (31)	25 (29)	n.s.

n.s.=not significant.

Table II. Results of a prospective, randomized, study comparing hCG, hCG and progesterone (P), and progesterone alone for luteal phase support in two OHSS risk groups (64)

	Low risk			High risk		
Group	Group I (hCG)	Group II (hCG + P)	Group III (P)	Group IV (hCG + P)	Group V (P)	Total
No. of patients	77	62	70	83	121	413
Estradiol concentration on day of hCG (pg/ml)[1]	1305±533	1202±576	1408±590	2534±896[2]	2677±984	1953±1018
OHSS °III	2.6	1.6	1.4	4.8	2.5	2.7
No. of oocytes obtained	7.19±2.58	6.16±3.41[3]	7.44±3.00	14.39±4.31	14.69±4.80	10.72±5.42
Embryos/transfer[4]	2.61±0.67	2.39±0.82	2.57±0.73	2.77±0.55	2.88±0.37	2.68±0.63
Clinical ongoing pregnancy rate per embryo transfer (%)	14.3	14.5	11.4	21.0	21.5	17.4

[1] $p<0.01$ low- *vs* high-risk group.
[2] No estradiol values were available for two patients in this group.
[3] $p<0.05$ for the number of oocytes obtained (Group II *vs* Group I and Group III).
[4] $p<0.05$ low- *vs* high-risk group.

- hCG (5000 IU) on the day of embryo transfer in combination with vaginal progesterone, 600 mg/day.

The results are presented in Table II. The results clearly show that, despite a higher pregnancy rate in the high-risk group – probably related to a better response among these patients – the pregnancy rates were not statistically significantly different within the risk groups. However, the incidence of OHSS was higher in the high-risk patients who received hCG. Therefore, in the absence of any advantage to achieving an ongoing pregnancy and an increased risk of OHSS, these data also confirm that the use of hCG either alone or in combination with progesterone offers no benefit over the use of vaginal progesterone alone.

In summary, there has been no study to date which has been able to show that luteal phase support with hCG is superior to that provided by progesterone. Due to the same efficacy of hCG and progesterone, but the increased OHSS risk with hCG, progesterone should be the first choice for luteal phase support following ovarian stimulation in the long protocol. The question is, by what route should progesterone be given. In addition to oral preparations, progesterone is also available as intramuscular and vaginal formulations. The various options have been compared in prospective, randomized studies.

Oral progesterone for luteal phase support

After oral administration, progesterone is broken down into numerous metabolites, which have unwanted effects on the uterus. Chromatographic studies show that the plasma concentration of non-metabolized progesterone falls below the level of detection just a few hours after oral administration (29). The metabolites formed are mainly 5α-reduced substances which exert a negative effect on the central nervous system (CNS) and on the uterus, as a result of binding to γ-aminobutyric acid (GABA) receptors (30). The metabolites have a sedative effect on the CNS, similar to that produced by benzodiazepines (31). Radioimmunoassay (RIA) can be used to measure the concentrations of these progesterone metabolites and the presence of these metabolites in high concentration after oral but not vaginal administration (32, 33). A direct, prospective, randomized comparison of vaginal and oral progesterone showed that there was a greater incidence of side-effects, e.g. sedation, after oral administration (34).

In a study by Nahoul et al. (33), it also was apparent that serum levels following vaginal administration of progesterone (100 mg) remained constant between 4 and 5 ng/ml for more than 24 hours, whereas progesterone levels after oral administration rose briefly to 1.5 ng/ml, but then fell sharply after a few hours. After 6 hours, progesterone levels were below 0.5 ng/ml (33).

In a prospective, randomized study, Pouly et al. (34) compared vaginal progesterone gel (90 mg/day) with oral progesterone, 300 mg/day, given in the form of Utrogestan® capsules (one in the morning, two in the evening). Ovarian stimulation for IVF was carried out according to a long protocol using the GnRH agonist, triptorelin, in a depot formulation, and hMG. A total of 283 patients with normo-ovulatory cycles whose infertility was caused by tubal factors or endometriosis were prospectively randomized after embryo transfer. Luteal phase support was given for 14 days or for 30 days if pregnancy occurred. There was no difference between the two protocols in terms of clinical efficacy (Table III).

Friedler et al. (35) carried out a prospective, randomized study involving 64 cycles with male factor

Table III. Outcome of a prospective, randomized study comparing vaginal progesterone (Crinone® 8%) and oral progesterone, 300 mg/day (Utrogest®) (34)

	Crinone® 8%	Utrogest®	Significance value
Embryo transfers	139	144	
Number of clinical pregnancies (%)	40 (28.8)	36 (25.0)	n.s.
Number of ongoing pregnancies (%)	36 (25.9)	33 (22.9)	n.s.
Number of births (% per transfer)	32 (23.0)	32 (22.2)	n.s.

n.s.=not significant.

infertility, with an estradiol level of at least 2500 pg/ml on the day of hCG administration and at least two embryos (35). The patients were given either Utrogestan® capsules vaginally (100 mg, twice daily) (n=32) or orally (200 mg, 4×daily) (n=32), starting on the day after embryo transfer and continuing up to 14 days thereafter. Ovarian stimulation was performed according to a long protocol using a depot formulation of triptorelin and hMG. The implantation rate was significantly lower in the group given oral progesterone (10.7% *vs* 30.7%; $p<0.01$, Fisher's exact test). The ongoing clinical pregnancy rate – i.e. excluding miscarriages – was 20.0% in the group given oral progesterone and 41.1% in the group given vaginal progesterone, though the difference between the groups was not significant.

In another prospective, randomized study, Licciardi et al. (36) compared intramuscular progesterone (50 mg/day) with oral progesterone (600 mg/day) beginning 2 days before embryo transfer (36). Ovarian stimulation was performed according to a long protocol with follicle-stimulating hormone (FSH), hMG or a combination of both. Only 43 patients were randomized, as the study was terminated early for ethical reasons – the implantation rate was significantly better in the group given intramuscular progesterone (40.9% *vs* 18.1%; $p<0.004$). The results are shown in Table IV.

On the basis of these data, and the fact that progesterone metabolites are found following oral administration, luteal phase support using oral progesterone should be avoided.

Intramuscular progesterone *vs* vaginal progesterone

Having established that oral progesterone should not be used, assessment of whether luteal phase support should preferentially be given via the intramuscular or vaginal route should be addressed. Other routes of administration, such as the rectal route, have not gained widespread acceptance and, to date, have not been evaluated in large, prospective, randomized studies (37).

To date, there has been only one prospective, double-blind, randomized, study comparing intramuscular and vaginal progesterone (Crinone® 8%) (38). In this study, 156 patients with tubal factor infertility received progesterone, 50 mg, i.m. or vaginal progesterone gel, 90 mg, as luteal phase support. A control group received intramuscular sodium chloride solution. Ovarian stimulation for IVF was performed according to a long luteal protocol. All luteal phase regimens were started on the day prior to embryo transfer and continued for up to 14 days after embryo transfer or, in the event of a pregnancy, up to week 14 of pregnancy. There was no difference between groups in plasma estradiol concentration during the luteal phase, however, progesterone concentrations in the treated patients were significantly higher than in the control group. Progesterone concentrations were also significantly higher among patients in the intramuscular progesterone group (42.5±13.0 *vs* 20.2±10.0 ng/ml) who also had a significantly higher rate of pregnancy than patients from either of the other two study groups (Table V). The authors, therefore, concluded that intramuscular progesterone provides better luteal phase support than vaginal progesterone.

Table IV. Outcome of a prospective, randomized, study comparing intramuscular progesterone (50 mg) and oral progesterone (600 mg). Values are means ± SEM unless otherwise indicated (36)

	Intramuscular progesterone	Oral progesterone	Significance value
Embryo transfers (n)	19	24	
No. of embryos transferred	3.47±0.193	3.46±0.170	n.s.
Mean embryo quality	1.86±0.099	1.91±0.072	n.s.
No. of clinical pregnancies/follicular punctures (%)	11/19 (57.9)	11/24 (45.8)	n.s.
Rate of multiple implantations/pregnancies (%)	9/11 (81.8)	4/11 (36.3)	n.s.
Implantation rate per transferred embryo (%)	40.9	18.1	$p<0.004$

n.s.: not significant.
Statistics using Mann–Whitney U-test, χ^2-test or Fisher's exact test.

Table V. Outcome of a prospective, randomized, double-blind study comparing intramuscular progesterone and vaginal progesterone (Crinone® 8%), and a control group without supplementation following the long protocol for IVF (38)

	i.m. progesterone	Vaginal progesterone	Control group[1]
Embryo transfers	52	52	52
Biochemical pregnancies (%)	45.7	30.6	12.5
Clinical pregnancies (%)[2]	34.3	19.1	6.8
Ongoing pregnancies (%)[3]	28.9	11.0	3.0
Live births (%)	22.1	8.0	2.8

[1] i.m. NaCl solution.
[2] Intrauterine amniotic sac or hCG above 1400 mU/ml, if no ultrasound was performed.
[3] Pregnancies which continued up to at least the 20th week of pregnancy.

While no other prospective, randomized, studies comparing i.m. and vaginal progesterone for luteal phase support have been published, data from non-prospective or non-randomized studies are available and are considered below.

In one study, 61 patients were analyzed retrospectively, having received ovarian stimulation according to a long protocol ($n=55$), a short protocol ($n=4$) or with clomiphene citrate and hMG ($n=2$) (39). A total of 49 patients received intramuscular progesterone (50–75 mg/day) and 11 patients received vaginal progesterone (Crinone® 8%; 90 mg daily). The authors reported an ongoing pregnancy rate of 32.6% in the intramuscular group compared with 0% in the vaginal group, indicating that vaginal administration of progesterone was significantly less effective ($p<0.005$). Although the authors claimed that intramuscular progesterone has a significant advantage over vaginal progesterone, these data are actually inconclusive due to the small numbers of patients involved in the study and to a certain degree of preselection that resulted in a poorer outcome for the patients receiving vaginal progesterone.

In 1999, Damario et al. (40) reported on a retrospective cohort study in which 44 patients were treated with vaginal progesterone (Crinone® 8%; 90 mg daily) (40). The data from these patients were compared with those from a historical population of 203 patients whose luteal phase had been supplemented with intramuscular progesterone (50 mg/day). Ovarian stimulation was performed according to a long protocol. The data from this study are presented in Table VI. The implantation rate was significantly worse in patients who received vaginal progesterone (26.2% *vs* 16.6%; odds ratio 0.56, 95% confidence interval 0.35–0.89). The ongoing pregnancy rate was also lower, though not significantly so, in this group (49.3% *vs* 34.1%; odds ratio 0.53, 95% confidence interval 0.27–1.04). The two groups were not entirely comparable, however, as the mean age of patients in the vaginal progesterone group was slightly higher than of those in the intramuscular group. Furthermore, these patients tended to require longer periods of ovarian stimulation with the use of significantly more ampoules of gonadotrophin (42.6 ± 20.4 *vs* 32.9 ± 14.7, $p<0.01$). It must be assumed that patients in the vaginal progesterone group were at an initial disadvantage that could easily have led to the lower implantation and pregnancy rates.

Both of these studies have methodological limitations and do not conclusively demonstrate that vaginal progesterone is less effective. In contrast, other better designed studies, which provide more reliable data, have not found any difference between these two routes of administration.

For example, Chantilis et al. (41) performed a prospective study in which a historical control group was included. In this study, 100 patients received vaginal progesterone (Crinone® 8%; 90 mg daily) and 106 received intramuscular progesterone, 50 mg/day, during the luteal phase. The stimulation protocols were similarly distributed in each group and, in most cases, followed the long protocol. The rate of ongoing clinical pregnancies was

Table VI. Comparison of vaginal progesterone (Crinone® 8%) and intramuscular progesterone in a retrospective study (40)

	Crinone® 8%	Progesterone i.m. (50 mg)	Statistics[1]
n	44	227	
Age (years)	34.1 ± 3.7	33.2 ± 4.0	$p=0.23$
Period of stimulation (days)	10.0 ± 1.3	9.7 ± 1.4	$p=0.08$
Number of gonadotrophin ampoules (75 IU)	42.6 ± 20.4	32.9 ± 14.7	$p<0.01$
Number of transferred embryos	3.30 ± 0.76	3.35 ± 0.78	$p=0.67$
Biochemical pregnancies (% per transfer)	15.9	5.7	3.11 (1.17–8.32)
Implantation rate (% per transferred embryo)	16.6	26.2	0.56 (0.35–0.89)
Clinical ongoing pregnancy rate (% per transfer)	34.1	49.3	0.53 (0.27–1.04)

[1] Statistics are expressed as values of *p* or as the odds ratio with a 95% confidence interval.
Values are means±standard deviation, unless otherwise stated.

Table VII. Results of a prospective observation study with Crinone® 8% in the USA (43)

	Crinone® 8%	SART Register 1997
n	1184	4801
Clinical pregnancy rates	35.1%	33.6%
Clinical ongoing pregnancy rates	31.0%	not reported

SART: Society of Assisted Reproductive Technologies (patients from the participating centers were included, patients supplemented with Crinone® 8% were excluded).

similar between groups – 32.0% (32/100) after vaginal and 34.9% (39/106) after intramuscular progesterone.

In another study, supplementation with 50 mg (≤40 years) or 100 mg (>40 years) progesterone i.m. daily was compared retrospectively with supplementation with Crinone® 8% once daily (≤40 years) or twice daily (>40 years) (42). Pregnancy rates of 25.7% (19/74) in the i.m. progesterone group and 29.5% (18/61) in the Crinone® 8% group were comparable.

In an extensive observational study in 16 IVF centres in the USA, supplementation with Crinone® 8%; 90 mg daily, was given over 1000 cycles for IVF (43). The results, shown in Table VII, when compared with the Society of Assisted Reproductive Technologies Register (SART), suggest that no differences result whether supplementation is administered with conventional protocols – particularly progesterone i.m. – or Crinone® 8%. However, the selection of patients for a prospective observational study might have a positive influence on pregnancy rates.

Direct questioning of patients, with previous experience of i.m. progesterone before the present cycle with Crinone® 8%, revealed that the overwhelming majority of those questioned rated Crinone® 8% as easier to administer, less painful and less time-consuming, and would prefer this preparation to the administration of i.m. progesterone in the subsequent cycle (43).

Finally, Schoolcraft et al. (44) recently reported their experience of the administration of vaginal progesterone in the form of Crinone® 8% (44). These data were compared with historical data and current treatment cycles with i.m. progesterone, and the results are presented in Table VIII. As in the study by Damario et al. (40), the group given vaginal progesterone had previously undergone significantly more IVF attempts (1.6±0.9 *vs* 0.7±0.8, $p<0.001$), and the stimulation period in days was significantly longer than in the i.m. progesterone group (9.7±1.2 *vs* 9.1±1.2, $p<0.027$). Nevertheless, the live birth rates per embryo transfer of 53.5% (vaginal progesterone) and 50.0% (i.m. progesterone) were comparable. These results correspond to the rates reported by SART for supplementation with i.m. progesterone (Table VIII). When the patients treated were questioned, it was also evident that most of those who had previous experience of i.m. progesterone considered use of the gel easier (9/13, 69.2%), less painful (10/13, 76.9%) and less time-consuming (8/13, 61.5%), with the majority stating a preference for vaginal progesterone to i.m. progesterone in a subsequent cycle (10/13, 76.9%) (44).

Further data on the vaginal administration of progesterone following IVF treatment highlight various advantages of this mode of administration. In 1990, a Belgian team demonstrated that a physiological, synchronous transformation of the endometrium took place only under the administration of vaginal progesterone and not when progesterone was given i.m. or orally. Despite transformation being observed following i.m. administration, the stroma and glands were not synchronized, and the glands tended to display a rigid structure and not the typical coiled structure (45). This may be because following vaginal administration, progesterone reaches the uterus directly, without passing through the liver. This *first uterine pass effect* was first described in 1995 and includes the observation that with low-peripheral progesterone serum levels, the concentration in the uterus can reach maximal values (46). This effect

Table VIII. Results of a prospective observation study with Crinone® 8%. This is a comparison of the data collected during this prospective trial and data from the SART Register with progesterone i.m. (44)

		Progesterone i.m.			
			SART Register 1998		
	Crinone® 8%	Own data of Schoolcraft et al. (44)	<35 years	35–39 years	>39 years
Embryo transfers (*n*)	43	46	164	147	93
Biochemical pregnancies (% per transfer)	31 (72.1)	34 (73.9)	–	–	–
Clinical pregnancies (% per transfer)	26 (60.5)	28 (60.9)	104 (63.4)	94 (63.9)	32.3 (30.0)
Births (% per transfer)	23 (53.5)	23 (50.0)	95 (57.7)	76 (51.7)	23.7 (22.0)

is not confined solely to the endometrium (45), but an effect on uterine activity can also be observed (47). Fanchin et al. (47) showed through M-mode sonography that, under the administration of progesterone, uterine contractions decreased as the levels of progesterone rose, and that this correlated, subsequently, with a higher implantation and pregnancy rate (47). The same team also showed that the rate of uterine contractions gradually decreased over a 7-day period after hCG administration to induce ovulation, i.e. on the day of embryo transfer. An average of 4.6 contractions/min was measured on the day of hCG administration, 3.5 contractions/min 4 days later and 1.5 contractions/min 7 days later (48). The conclusion that can be drawn from this is that optimal progesterone supplementation should begin on the day of oocyte retrieval or 1 day later – before embryo transfer on day 2. This is supported by the results from other studies which showed that in the uterus *ex vivo*, complete diffusion in the myometrium occurred within 6 hours of vaginal administration of progesterone (49). Apart from direct diffusion, an active mechanism may be involved in progesterone transport, as demonstrated by means of hysterosalpingography of the uterus and uterine tubes (50). Finally, *counter-current exchange* appears to be involved in progesterone transport in the course of utero-vaginal perfusion (51, 52). This term describes the process by which significantly higher concentrations of substances injected into an ovarian vein are detected in the ipsilateral artery than in the contralateral ovarian arteries or peripheral veins. This finding is attributed to the passage of substances along a concentration gradient established between arteries that are physically close to each other but which contain blood flowing in the opposite direction. This mechanism explains why there is an approximately 14-fold greater concentration of progesterone in the uterus compared with the periphery following vaginal administration; the ratio after i.m. administration tends to be around 1:1 (52).

On the basis of these data, vaginal rather than i.m. administration of progesterone mimics more closely a physiological form of endometrium transformation with a reduction in uterine activity or peristalsis, and consequently leads to an increased chance of implantation following IVF.

While a variety of retrospective or prospective uncontrolled studies have shown no difference between vaginal and i.m. administration of progesterone, in terms of clinical pregnancy rate, one prospective, randomized, and three retrospective studies have favored i.m. progesterone (53). However, the latter studies are methodologically flawed, making their outcome questionable. Thus, from the data available, the situation regarding any disadvantage of vaginal progesterone administration is far from proven.

Interestingly, studies relating to egg donation provided evidence of equivalent pregnancy rates following i.m. and vaginal progesterone administration in a prospective randomized study (54, 55). These data are of special importance because they showed, that in women without any inherent ovarian function and after preparation of the endometrium with transdermal estrogen, vaginal progesterone administration results in satisfactory endometrial transformation. In the first study, Crinone® 8% was given twice daily as the form of vaginal progesterone. The ongoing clinical pregnancy rates were comparably high, with 31% in the Crinone® 8% group and 22% in the progesterone i.m. group (100 mg daily) (54). This study was subsequently continued, with administration of Crinone® 8% being reduced to a single daily dose. Extracts from the results are reproduced in Table IX and provide unequivocal evidence of an equivalent pregnancy rate.

Nevertheless, there is an undoubted need for more extensive, prospective randomized studies to provide evidence of the equivalence of i.m. and vaginal administration of progesterone.

Comparison of different progesterone preparations for vaginal administration

A limited number of progesterone preparations are currently available that can be administered vaginally.

- Progesterone capsules (e.g. Utrogest®, Dr. Kade, Berlin, Germany) containing 100 mg natural progesterone per capsule can be used. The dose used in most studies is 600 mg/day, which therefore requires two capsules to be administered three times a day.
- A variety of progesterone pessaries are also available, the majority of which appear to have equivalent pharmacokinetic properties to progesterone capsules.
- Crinone® 8% vaginal gel (Serono International S.A., Geneva, Switzerland), a preparation that contains 90 mg natural progesterone, is available in an applicator specially developed for vaginal administration. Various studies have shown that 90 mg administered once daily seems to be at least as effective as 600 mg Utrogest® for luteal phase support in the course of IVF treatment.

The essential difference between progesterone capsules or suppositories and Crinone® 8% vaginal gel is that the former contains an oil emulsion, while the latter is an oil-in-water emulsion

Table IX. Results of a prospective, randomized study of the vaginal or i.m. administration of progesterone in the context of egg cell donation (55)

	Crinone® 8%	Progesterone i.m. (100 mg/day)
Cycles (*n*)	42	44
Mean endometrium thickness on cycle day 26 (mm)	10.3±2.8	10.0±3.2
Implantation rate per transferred embryo (%)	21.4 (34/159)	19.0 (30/158)
Ongoing clinical pregnancy rate beyond 20 weeks of pregnancy/transfer (%)	39.1 (18/46)	40.9 (18/44)

Values are means±standard deviation, unless otherwise stated.

Table X. Results of a prospective, randomized study comparing Crinone® 8% and Utrogestan® in the short protocol with hMG (56)

	Utrogestan®	Crinone® 8%
n	48	51
Age (years) (median)	31	32
Embryos/transfer (mean)	2.8	2.75
Estradiol on day of hCG admin. (mean±standard deviation)	776±835	651±489
Clinical pregnancies (% per embryo transfer)	18 (37.5)	18 (35.3)
Multiples (n)	4	3
Abortions	2	3

on a polycarbophil base. While the polycarbophil ensures that the preparation adheres to the vaginal epithelium, the oil-in-water emulsion guarantees the continuous release of progesterone directly from the aqueous phase. Replenishment of the hormone in the aqueous phase is with micronized progesterone from the depot oily phase.

In a prospective, randomized study, 99 short protocol IVF cycles were prospectively randomized to Utrogestan® or Crinone® 8% following ovarian stimulation with hMG (56). The results revealed no significant difference between the two groups in any parameter (Table X).

In our prospective, randomized study, 126 patients received luteal supplementation, administered by the vaginal route, with either two capsules of Utrogest® three times a day (600 mg/day) or 90 mg Crinone® 8% once daily. Administration followed stimulation with hMG or recombinant FSH after either the long protocol or the multiple dose antagonist protocol with Cetrotide® (Serono International S.A., Geneva, Switzerland). There were no differences between the groups either demographically or in relation to the method of stimulation used. The ongoing pregnancy rates of 24.7% in the Crinone® 8% group and 17.0% in the Utrogest® group were comparably high, and were not significantly different (Table XI).

Table XI. Outcome of a prospective, randomized study comparing vaginal progesterone (Crinone® 8%) and Utrogest® (65)

	Crinone® 8%	Utrogest®	
n	73	53	
Age (years)	31.41±5.52	31.45±4.29	n.s.
Previous treatment cycles for IVF or IVF/ICSI	1.07±1.15	1.04±1.14	n.s.
Previous pregnancies	0.51±0.80	0.58±0.82	n.s.
Percentage primary sterility (%)	64.2	68.5	n.s.
Cycles with recFSH (%)[1]	68.5	75.0	n.s.
Long protocol (%)	64.4	77.4	n.s.
Multiple-dose GnRH-antagonist protocol (%)	35.6	22.6	n.s.
Estradiol (pg/ml)	2646±1369	2712±1540	n.s.
No. of egg cells	12.67±5.01	13.94±5.93	n.s.
No. of transferred embryos (mean±s.d.)	2.78±0.45	2.77±0.47	n.s.
Cumulative embryo score (mean±s.d.)	25.55±10.44	23.60±10.90	n.s.
Clinical pregnancies (pos. heart actions) (%)	21 (28.8)	10 (18.9)	n.s.
Clinical abortions up to 12th week of pregnancy (%)	3 (15.8)	1 (10.0)	n.s.
Ongoing clinical pregnancies (%)	18 (24.7)	9 (17.0)	n.s.

n.s.: not significant; s.d.: standard deviation.

[1] All other cycles were stimulated with hMG.

Statistics using Mann–Whitney U-test or χ^2-test.

In addition, 47 patients selected at random, who had not become pregnant in the course of this study, were questioned about their satisfaction with the luteal phase support. It appeared that fewer patients had difficulties administering Crinone® 8% than Utrogest® ($p<0.05$), and complained less frequently of vaginal discharge ($p<0.01$). Patients who already had experience of either Crinone® 8% or Utrogest® from an earlier cycle and had been treated in this study with the other of the two preparations, considered Crinone® 8% less time-consuming ($p<0.05$) and easier to handle ($p<0.01$) when compared directly with Utrogest® (65).

Levine (43) supports these findings, in an interim analysis of a prospective study of Crinone® 8%. Over 1000 IVF patients were questioned regarding their satisfaction with the preparation. Patients who had experience of vaginal suppositories or a comparable preparation from an earlier cycle considered Crinone® 8% to be less time-consuming, easier to handle and less painful in use, and would prefer it to Utrogest® in a subsequent cycle. However, it should be noted that a potential source of error in the analysis arises from the fact that *all* patients were receiving Crinone® 8% in the current cycle, and no distinction was made between pregnant and non-pregnant patients. Thus, while a certain data bias cannot be ruled out, these findings support the results of our own study.

To summarize, these data clearly indicate that the specific development of Crinone® 8% for vaginal administration offers unequivocal advantages over the use of capsules or suppositories. Given their equivalent clinical efficacy, preference should be given to Crinone® 8% when choosing a vaginal progesterone preparation because patients prefer it.

Supplementary administration of estrogens for luteal phase support

Controversy surrounds the benefits derived from supplementation of endogenous estrogen levels for luteal support. Three prospective, randomized studies of this practice have been conducted.

In an extensive study, 378 patients, stimulated with hMG according to a long protocol, were randomized to receive no estrogen supplementation, or supplementation with 6 mg estradiol valerate during the course of luteal phase support with 600 mg vaginal progesterone daily. The pregnancy rates recorded in both groups were identical – 29% (4).

Similar findings were reported by Lewin et al. (57), who randomized 100 consecutive patients to receive either supplementary administration of 2 mg estradiol valerate daily or no supplementation after stimulation in the long protocol under luteal phase support with 50 mg progesterone i.m. daily (57). No significant differences existed between the two groups either in respect of pregnancy rates per embryo transfer (28.0% with estradiol *versus* 26.5% without estradiol supplementation) or in respect of birth rates per pregnancy (78.6% with estradiol *versus* 76.1% without estradiol supplementation). The supplementary administration of estradiol was, therefore, found to be of no benefit.

In a third study, patients with an estradiol level above 2500 pg/ml on the day of hCG administration were randomized to placebo or supplementary administration of 2 mg estradiol under luteal phase support with 150 mg of either i.m. or vaginal progesterone per day. Analysis of cycles conducted following the long protocol showed that the pregnancy rates following supplementary administration of estradiol were 39.6% (40/101) compared with 25.6% (29/113) in those given placebo, and were statistically significantly different ($p<0.05$) (58).

Use of the long protocol in the placebo group was considered a possible cause of the difference in outcome of the study compared with the studies by Lewin et al. (57) and Smitz et al. (4). However, as the long protocol was used in all three studies, this line of argument is unconvincing. Nevertheless, it is interesting that in the study by Farhi et al. (58), the patients were not originally stratified according to the protocols used (short and long protocol) and, therefore, the conclusions reached are based on a retrospective separation of the data. As data relating to the patients included are not reported, the possibility that the retrospective separation of the data produced some inhomogeneity in the groups that influenced the success of the supplementary treatments cannot be excluded.

Overall, however, supplementary administration of estradiol for luteal phase support appears unnecessary, although the data are inconsistent. Further studies on endogenous estradiol values would undoubtedly be worthwhile to more thoroughly investigate this approach.

Efficacy of ongoing luteal phase support in early pregnancy

At the beginning of a pregnancy, the endometrium and embryo continue to receive progesterone from the corpora lutea. The luteoplacental shift, when progesterone production is taken over by the developing placenta, does not take place until about the 8th–10th week of pregnancy. Evidence supporting the occurrence of the luteoplacental shift came from animal experiments in which the corpus luteum was removed in early pregnancy. Spontaneous

Table XII. Results of a prospective, randomized study of hormonal supplementation in early pregnancies after stimulation for IVF. Stimulation achieved with clomiphene citrate, hMG or hMG in the long protocol (63)

	Administration of 17α-hydroxy-progesterone (500 mg) and estradiol valerate (10 mg) i.m. (Gravibinon®, Schering, Berlin) twice weekly	No substitution	
Pregnancies (*n*)	55	65	
Age (mean±s.d.)	31.7±0.7	32.8±0.7	n.s.
Stimulation protocols			
– clomiphene citrate	2	3	
– clomiphene citrate/hMG	14	17	
– hMG	25	27	
– long protocol	14	18	
Estradiol on day of hCG admin. (pg/ml) (mean±s.d.)	3174±340	2767±314	n.s.
Biochemical pregnancies (%)	1 (2)	17 (27)	$p<0.001$
Clinical abortions (%)	5 (9)	9 (14)	n.s.
Ongoing clinical pregnancies beyond the 12th week of pregnancy (%)	48 (89)	38 (59)	$p<0.01$

Mean±s.d.: mean±standard deviation.
n.s.: not significant.

abortions occurred when the lutectomy was performed before the 7th week of pregnancy, whereas the pregnancy proceeded normally when the operation was delayed. Moreover, measurement of estradiol and progesterone levels showed that concentrations of these hormones decreased after a lutectomy, but subsequently increased in those cases in which a spontaneous abortion did not occur (59). The authors also showed that the deleterious effect of a lutectomy before the 7th week of pregnancy could be avoided by supplementation with progesterone (60). On the basis of the results of these studies, the authors postulated the phenomenon of the luteoplacental shift, and commented that the embryo is itself capable of maintaining an adequate progesterone concentration beyond a certain point in early pregnancy. Other studies determined the proportion of progesterone production that occurs in the ovaries. In the 6th week of pregnancy this is 75%, and it falls in the 10th and 15th week of pregnancy to 50% and 25%, respectively (61). More recent studies confirmed that there is a marked increase in the placental production of progesterone after the 8th week of pregnancy and, therefore, the luteoplacental shift begins at this point in time (62).

As luteal phase insufficiency obviously merits supplementation therapy after ovarian stimulation in the long protocol, as the above studies show, hormonal support during the early stages of pregnancy is indicated. However, with regard to the continuation of luteal phase support beyond this early phase with progesterone or other hormones, to date, only one prospective randomized study has been reported. This study reports on the administration of 17α-hydroxyprogesterone caproate (500 mg) and estradiol valerate (10 mg) twice a week i.m. in the form of the drug Gravibinon® 2 ml (Schering AG, Berlin, Germany) (62).

One hundred and twenty pregnant patients were prospectively randomized during early pregnancy to receive either Gravibinon® or no supplementation up to the completion of the 12th week of pregnancy following ovarian stimulation in the long protocol for IVF. The stimulation protocols were heterogeneous, and included those with clomiphene citrate alone, clomiphene citrate combined with hMG, hMG alone and hMG in the long protocol.

Administration of progesterone up to the time clinical pregnancy was detected significantly reduced the rate of subclinical abortions (Table XII). Continued administration beyond this time produced no further apparent benefit.

However, these outcomes require confirmation in further studies as patient numbers were low and stimulation protocols were heterogeneous. A prospective and randomized study design should investigate not only how long continuing support in early pregnancy should be given, but also, whether additional supplementation with progesterone is beneficial.

Summary

From the data presented here, the following conclusions may be drawn concerning the practice of luteal phase support in IVF following ovarian stimulation according to the long protocol:

- in principle, luteal phase support is necessary to optimize the results of treatment;
- luteal phase support with hCG is not superior to luteal phase support with progesterone;
- supplementary administration of hCG brings

no advantage when progesterone is administered;
- luteal phase support with hCG brings an increased risk – as compared with luteal phase support with progesterone – of OHSS and its corresponding potential complications;
- the administration of estradiol to supplement luteal phase support is probably not worthwhile, although a definitive conclusion cannot be drawn due to the controversial nature of the data available;
- the use of oral progesterone is clearly inferior to i.m. or vaginal administration, and is associated with an increased rate of side-effects due to unphysiological metabolites;
- at present, insufficient data are available for a direct comparison of i.m. with vaginal progesterone, but there is no reason to expect a disadvantage with vaginal progesterone administration.

In general, physiological endometrial transformation and adequate calming of any myometrial activity would be expected following vaginal administration of progesterone, potentially optimizing implantation rates following embryo transfer. Endometrial transformation, in particular, follows a more physiological 'natural' course after vaginal rather than i.m. administration of progesterone. In addition, optimal progesterone levels are achieved in the uterus following vaginal administration due to the uterine *first pass* effect; progesterone levels are 14-fold higher in the uterus than in the periphery following vaginal administration, whereas progesterone levels are maximally increased in both the uterus and the periphery following i.m. administration.

On the basis of these observations, the administration of vaginal progesterone for luteal phase support appears to be the optimal protocol at present. A comparison of various vaginally administered progesterone preparations shows that once-daily administration of the vaginal gel Crinone® 8% is superior to multiple daily administration of capsules or suppositories in terms of patient satisfaction.

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