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A prospective randomized study comparing intramuscular with intravaginal natural progesterone in programmed thaw cycles

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A simple programmed thaw cycle is described, during which the endometrium is prepared with oral oestradiol, followed by a natural progesterone source. This method involves minimal blood tests and uses inexpensive medications. Originally, an i.m. progesterone-in-oil preparation was used. Although highly successful in achieving high serum progesterone concentrations, the daily injections of the oily compound were found to be problematic from the patients' perspective. To examine the possibility of replacing the injectable progesterone with a vaginal preparation we conducted a prospective randomized study of 1 year's duration, during which time 170 and 184 thawing cycles were done with injectable and vaginal progesterone respectively. Although the progesterone blood concentrations obtained with the injectable preparation were more than twice those obtained with the vaginal progesterone, the clinical pregnancy rates (defined as percentage thawing cycles with gestational sac(s)/embryo transfer as demonstrated on ultrasound, 4 weeks after embryo transfer) were similar for both groups (15.9 and 16.8% respectively). Implantation and abortion rates were also comparable. These results agree with previous reports of higher uterine progesterone concentrations after the vaginal application. We conclude that the combination of oral oestradiol and vaginal progesterone is an effective, simple and inexpensive approach for programmed thaw cycles.

Key words: cryopreservation/human embryos/natural progesterone/thaw cycle/vaginal route of administration

Introduction

Continuing efforts to curb multifetal pregnancy rate in in-vitro fertilization (IVF) result in a trend to limit the number of transferred embryos to two (Templeton and Morris, 1998). This trend results in an increasing number of 'spare' embryos that can be cryopreserved for future use in thaw cycles. In fact, an efficient embryo cryopreservation programme should be an integral part of any assisted reproductive technology service.

The outcome of a thaw cycle relies on several factors: quality of the embryos that were cryopreserved, laboratory freezing and thawing procedures, uterine receptivity on the day the thawed embryos are transferred and adequate luteal support. Uterine receptivity depends on the way the endometrium was prepared for embryo transfer. Basically, three methods are used for endometrial preparation: natural cycle, programmed cycle, ovarian stimulation cycle. Based on our data (Lightman *et al.*, 1997), and that of others (Sathanandan *et al.*, 1991; Pattinson *et al.*, 1992), these three approaches result in comparable pregnancy rates. Since the programmed cycle method described (Serhal and Craft, 1987) for ovum donation is the simplest approach as far as the patient is concerned, our general policy is to choose this method. The major advantages of this approach are that prior gonadotrophin-releasing hormone agonist (GnRHa) suppression is not needed (Lelaidier *et al.*, 1992, 1995; Pattinson *et al.*, 1992; Queenan *et al.*, 1997; Simon *et al.*, 1998), and that there is full control of the timing of embryo transfer.

Typically in these cycles, progesterone is given as i.m. injections of natural progesterone in oil (Queenan *et al.*, 1997). This route of administration of an oily preparation is not met with great enthusiasm by most patients, as it is often associated with painful injections and rash. Therefore, we sought an alternative preparation that can supplement the required progesterone. Based on prior data (Smitz *et al.*, 1992), it appears that vaginal progesterone may present such a desirable alternative. These researchers compared i.m. and intravaginal luteal phase supplementation during 'fresh' IVF cycles using fresh embryos. Despite lower plasma progesterone concentrations, the outcome in the group receiving intravaginal progesterone was as favourable as that in the group receiving i.m. progesterone. Similar results were described in stimulated cycles (Bourgain *et al.*, 1994), and in a donor oocyte programme (Gibbons *et al*, 1998). However, other researchers recommend the use of injectable progesterone based on better outcome compared to vaginal progesterone (Perino *et al.*, 1997).

The purpose of the current study, therefore, was to compare, in a prospective randomized fashion, the outcome of programmed freeze–thaw cycles during which either i.m. or vaginal progesterone was used.

Materials and methods

Patients

From January 1998 to December 1998, 354 programmed thaw cycles were performed at the Rambam Medical Center, Haifa, Israel. On cycle days 2–4 baseline serum oestradiol, progesterone and luteinizing hormone (LH) concentrations were taken to ascertain early follicular phase. Exogenous oestradiol was started with a dose of 6 mg

Table I. Uterine preparation protocol and randomization procedure

Day relative to embryo transfer	Cycle day	Oestradiol (mg/day)	Progesterone		Procedure
			Utrogestan (mg)	Gestone	
	2–4	6	–	–	Randomization Hormone analysis
–	↓10–20	6			
–2		4	600	50	Hormone analysis[a]
–1		4	600	100	
0	Embryo transfer	4	600	100	Embryo transfer[b] Hormone analysis
+	↓	4	600	100	
	12 days after embryo transfer	4	600	100	β-HCG[c] Hormone analysis

[a]Blood sample for hormone concentrations is taken before progesterone is given.
[b]In this particular example embryos cryopreserved on day 2 are transferred.
[c]Treatment continues if pregnancy is established by positive β-HCG.

(Estrofem; Novo Nordisk, Bagsvaerd, Denmark) for 12–20 days. Repeated serum oestradiol and progesterone analysis was done prior to progesterone initiation to rule out endogenous progesterone secretion (following spontaneous ovulation) and to ascertain patient compliance. Patients were randomly allocated to either i.m. (Gestone; Paines and Byrne, UK, 100 mg daily), or vaginal (Utrogestan, Besins Iscovesco, France, 200 mg three times daily) progesterone. Randomization was allocated by the last digit of the patient i.d. number: odd numbers were assigned to vaginal, even numbers to i.m. progesterone. Oestradiol dose was reduced to 4 mg daily on the day progesterone was added. Embryo transfer was done on the third or fourth day of progesterone treatment, according to the embryo age: embryos frozen on the second and third day post-retrieval were transferred after 48 h and 72 h exposure to progesterone respectively. Twelve days after embryo transfer, hormone analysis (oestradiol, progesterone, LH) was done together with β-human chorionic gonadotrophin (β-HCG) determination. If serum concentrations for progesterone were reported as >127 pmol/l, the value for analysis was taken as 127. If pregnancy was confirmed by a positive β-HCG, hormonal treatment (Estrofem 4 mg daily and the progesterone source) was continued until completion of the ninth gestational week. The ninth gestational week was chosen to discontinue progesterone treatment because a luteal–placental shift occurs at 7–8 weeks gestation, and prolonged progesterone administration may have a teratogenic effect on a developing fetus. Schematic presentation of the protocol used is given in Table I.

Embryo quality grading

The classification method was based on a modification of the grading system described by Veeck (1990). Briefly, embryos were graded on a scale of 1–5, where grade 1 embryos have barely defined blastomeres with major or complete fragmentation; grade 2 embryos have irregular or uneven blastomeres with moderate to heavy fragmentation; grade 3 embryos have uneven blastomeres with minor fragmentation; grade 4 embryos have regular and even blastomeres with minor fragmentation; and grade 5 embryos have regular and even blastomeres and no fragmentation.

Freezing and thawing protocols

Embryo freezing and thawing were performed as previously published (Lightman *et al.*, 1997). Briefly, day 2 or 3 embryos were exposed to a graded series (0.5, 1, 1.5 mol/l) of propanediol (PROH) in modified Dulbecco's phosphate-buffered saline (Sigma, St Louis, MO, USA) or HEPES-HTF (Irvine Scientific, Irvine, CA, USA) with 15% Synthetic Serum Substitute (Irvine Scientific). Embryos were then loaded into Nunc cryovials (Nunc, Roskilde, Denmark) containing 1.5 mol/l PROH and 0.2 mol/l sucrose and frozen in the Planer freezer (Planer Products, London, UK). Embryos were cooled from room temperature to –6.5°C at 2°C/min, held for 10 min after seeding, and cooled to –30°C at 0.3°C/min. After holding for 5 min at –30°C, embryos were cooled to –180°C at 50°C/min and plunged into liquid nitrogen for storage. For thawing, the vials were immersed in a 37°C water bath for a few minutes until all ice disappeared. The cryoprotectant was then removed in a stepwise fashion by decreasing the propanediol concentrations at 5 min intervals. The embryos were then evaluated, graded and transferred after 1–4 h in culture.

Clinical pregnancy confirmation

A pregnancy test was done 12 days after embryo transfer. If positive, transvaginal ultrasound was performed 2 weeks later to confirm clinical pregnancy [defined as demonstrable gestational sac(s)].

Statistics

Statistical analysis of the results was carried out using Student's *t*-test and χ^2 analyses The 5% significance level was considered as significant.

Results

A total of 354 thaw cycles was performed. In 170 and 184 cycles i.m. Gestone and vaginal Utrogestan were used, respectively. Both groups were comparable in age at embryo freezing and thawing, and in various clinical aspects as shown in Table II. Intuitively, embryos obtained and cryopreserved during cycles that have resulted in clinical pregnancy (either from fresh transfer, or from a subsequent thaw cycle) have a higher chance of achieving pregnancy. Our two treatment groups were also comparable in that regard (Table II).

Table III summarizes the relevant clinical findings in the two groups. Both groups were treated with Estrofem for a mean period of 15 days before progesterone was added (either Gestone or Utrogestan). The concentrations of oestradiol, progesterone and LH, taken before progesterone was added, were comparable in the two groups.

Oestradiol concentrations on the day of β-HCG determination were significantly higher in the vaginal group. Of note is

Table II. Demographic and clinical characteristics of the two population groups

	Progesterone	
	Gestone	Utrogestan
Number of thawing cycles	170	184
Age at embryo freezing (mean ± SD)	30.4 ± 5.4	31.3 ± 5.5
Age at thaw cycle (mean ± SD)	31.5 ± 5.4	32.0 ± 5.6
Diagnosis		
Male	106	115
Mechanical	20	38
Unexplained	33	16
Other	11	15
Previous pregnancy		
Fresh IVF embryos	34	36
Frozen IVF embryos	26	24

the highly significant difference in progesterone concentrations on day of β-HCG between the two groups. The higher progesterone concentrations in the i.m. group were not associated with higher pregnancy rate. The vaginal group had a 16.8% clinical pregnancy rate versus 15.9% for the i.m. group. This difference was not significant by χ^2 analysis. Implantation rates for both groups were almost identical (6.9 versus 7.0%). The mean number of transferred embryos was identical in both groups. The sum of individual embryo grading in each embryo transfer was taken to reflect the quality of the transferred embryos. The two groups were also nearly identical in this regard. The vaginal group had a higher first trimester abortion rate (26 versus 11%), but this difference was not statistically significant.

Discussion

The notion that high circulating progesterone concentrations during the luteal phase are advantageous has been suggested (Sauer *et al.*, 1991) in the oocyte donation setting. This conclusion, based on histological evidence, was not supported by other researchers. Looking at endometrial maturation after stimulated cycles, Bourgain *et al.* observed no significant differences in the ultrastructural features, or the oestrogen and progesterone receptor distribution of the endometrium, after i.m. or intravaginal progesterone supplementation (Bourgain et al., 1994). If there was a difference at all, it was that after intravaginal progesterone administration, the endometrium was more frequently in phase with respect to the cycle day, despite lower plasma progesterone concentrations (Bourgain *et al.*, 1990; Smitz *et al.*, 1992; Miles *et al.*, 1994; Fanchin *et al.*, 1997; Gibbons *et al.*, 1998). It was therefore suggested that plasma progesterone concentrations do not necessarily reflect local endometrial concentrations, which might be higher after using the vaginal route of administration. Direct endometrial progesterone concentration measurements were in agreement with this suggestion (Miles *et al.*, 1994). They showed that intravaginal administration of micronized progesterone resulted in an 8-fold increase in endometrial progesterone concentration compared with i.m. administration, despite a 3-fold decrease in serum progesterone concentration. Moreover, the existence of a 'first uterine pass effect' when drugs are delivered

Table III. Results of thaw cycles: mean serum hormone concentrations, number of embryos transferred and their grading and cycle outcome. Data represent mean ± SD. NS = not statistically significant

	Progesterone administration		*P*-value[a]
	i.m. (n = 170)	Vaginal (n = 184)	
Days of oestradiol only	15.3 ± 3.9	15.6 ± 3.7	NS
Oestradiol (pmol/l) before progesterone	847 ± 385	861 ± 395	NS
Progesterone (nmol/l) before progesterone	1.5 ± 1.3	1.8 ± 2.5	NS
LH (IU/l) before progesterone	10.2 ± 6.4	10.2 ± 6.9	NS
Oestradiol on day of β-HCG determination	581 ± 267	651 ± 277	0.02
Progesterone on day of β-HCG determination	108 ± 28.7	47.5 ± 29.7	<0.0001
Total no. of embryos transferred	481	526	–
No. of embryos	2.9 ± 0.8	2.9 ± 0.8	NS
Sum of embryo grading	8.3 ± 2.5	8.4 ± 2.6	NS
Total no. of implantations	33	37	–
Implantation rate (%)	6.9	7.0	NS (χ^2)
Clinical pregnancies (% of embryo transfers)	27 (15.9)	31 (16.8)	NS (χ^2)
No. of early abortions[b] (% of clinical pregnancies)	3 (11)	8 (26)	NS (χ^2)

[a]By *t*-test, except as noted.
[b]All abortions occurred before 9 weeks gestation.

vaginally has been clearly demonstrated (Bulletti *et al.*, 1997), explaining the endometrial/plasma progesterone concentration differences. Hence, the vaginal route offers the advantage of targeted drug delivery to the uterus. In programmed thaw cycles the endometrium is the only target for progesterone delivery; therefore these data (Bulletti *et al.*, 1997) establish the mechanism for using the vaginal route to deliver progesterone in these cycles.

Our data seem to be in agreement with this line of evidence. Despite significantly lower plasma progesterone concentration, vaginal progesterone resulted in a pregnancy rate comparable to that achieved with i.m. progesterone. On the other hand, higher pregnancy rates have been reported with injectable progesterone compared with micronized vaginal progesterone used for luteal phase support (Perino *et al.*, 1997). It should be noted that Perino and coworkers used only one-third of the vaginal progesterone dose used in our study (i.e. 200 mg/day versus 600 mg/day). This significant difference may explain the less favourable outcome reported.

Although the two routes of administration did not differ significantly in terms of abortion rate (Table III), the numbers of patients in each group were quite small. Further studies with larger numbers of patients are required to confirm this lack of association.

Being an open study, our non-biased randomization was strictly enforced as evidenced by the similarity between the two groups in the relevant clinical aspects (age at freezing and thawing, diagnosis, number of embryos replaced and their quality etc.). We also ruled out a possible bias of using embryos originating from a successful previous fresh cycle. The strategy in planning programmed thaw cycles should be based on success rate, patient comfort and cost. Using prior GnRHa

suppression increases the cost considerably, but does not contribute to higher success rate (Simon *et al.*, 1998). Injectable progesterone in oil is clinically acceptable, although we frequently observe disturbing side-effects (local reaction, fever, urticaria) after its use. Patients often express their desire to have an alternative treatment, especially as extended progesterone supplementation in these cycles is imperative if pregnancy is to be achieved. Oral micronized progesterone is a valid option, although the direct endometrial effect does not take place, and its bioavailability is only 10% of that found after i.m. progestrone (Simon *et al.*, 1993). Moreover, patients receiving oral progesterone often complain of disturbing side effects, mainly drowsiness. Based on these considerations, we suggest that a programmed cycle based on vaginal progesterone, as described herein, is not only simple and successful, but also more convenient to the patients. The apparently alarming low plasma progesterone concentrations achieved by the vaginal route should be regarded in the light of the probable higher endometrial concentrations.

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