

# Uterine contractility decreases at the time of blastocyst transfers

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**High-frequency uterine contractions at the time of non-cavitating embryo transfer influence adversely IVF–embryo transfer outcome. This prompted us to quantify prospectively the possible decline in uterine contraction frequency occurring during later stages of the luteal phase of ovarian stimulation, up to the time of blastocyst transfers, in 43 IVF–embryo transfer candidates. Contractility was assessed on the day of human chorionic gonadotrophin (HCG) administration, 4 days after HCG (non-cavitating embryo transfer; HCG + 4), and 7 days after HCG (blastocyst transfers; HCG + 7). For this, 2 min sagittal uterine scans were obtained by ultrasound and digitized with a computerized system for the assessment of uterine contraction frequency. Our results indicated that a slight, yet significant, decrease in uterine contraction frequency, observed from the day of HCG ( $4.4 \pm 0.2$  contractions/min) to HCG + 4 ( $3.5 \pm 0.2$  contractions/min), was followed by a more pronounced, additional decrease between HCG + 4 and HCG + 7 ( $1.5 \pm 0.2$  contractions/min;  $P < 0.001$ ). In conclusion, during the luteal phase of ovarian stimulation, uterine contractility decreases progressively, and reaches a nearly quiescent status 7 days after HCG administration, at the time of blastocyst transfers. It is possible that such a uterine relaxation assists blastocyst implantation.**

*Key words:* blastocyst/embryo implantation/ultrasound/uterine contractions

## Introduction

Growing evidence indicates that blastocysts have greater implantation potential compared with non-cavitating embryos (Gardner *et al.*, 1998a,b; Ménézo *et al.*, 1998; Milki *et al.*, 1999). Today, implantation rates of blastocysts often approximate 50% (Gardner *et al.*, 1998a,b; Schoolcraft *et al.*, 1999), which allows a reduction in the number of transferred embryos and, consequently, in the risk of high order multiple pregnancies. These data concur to make blastocyst transfers one of the most attractive improvements of modern IVF–embryo transfer. The explanations primarily proposed for the outstanding results obtained with this technique include the selection of the healthiest embryos by an extended culture, and/or the improvement of endometrial receptivity on the fifth day after fertilization.

Yet recent studies of uterine contractility in IVF–embryo transfer led us to consider an alternative, and possibly complementary, explanation for the high implantation rates of blastocysts. It has been demonstrated that myometrial contractile activity influences embryo implantation, possibly through mechanical displacement of embryos, in both animals (Pusey *et al.*, 1980; Rogers *et al.*, 1983) and humans (IJland *et al.*, 1997, 1998; Fanchin *et al.* 1998). Data on the relation

between uterine contractility and embryo implantation in humans remained scarce because of the invasiveness of traditional methods that required the introduction of pressure probes into the uterine cavity (Henry *et al.*, 1943; Hendricks, 1966; Martinez-Gaudio *et al.*, 1973).

However, the advent of high resolution ultrasound probes permitted the direct visualization of myometrial activity in ultrasound scans (Oike *et al.*, 1990; Abramowicz and Archer, 1990; Lyons *et al.*, 1991; IJland *et al.*, 1997, 1998). Recently, the reliability of the ultrasound approach for assessing uterine contractions was confirmed by demonstrating the concordance of findings made simultaneously on ultrasound and intrauterine pressure recordings in an experimental model (Bulletti *et al.*, 2000).

In earlier investigations (Fanchin *et al.*, 1998, 2000a), we have shown that a considerable fraction of IVF–embryo transfer patients have persistently high uterine contraction frequency at the time of non-cavitating embryo transfer, 4 days after human chorionic gonadotrophin (HCG) administration. Moreover, the observation of high-frequency uterine contractions ( $>5.0$  contractions/min) at the time of embryo transfer is associated with markedly lower implantation and pregnancy rates per embryo transfer (4 and 14% respectively) as compared with cases with low-frequency contractions ( $\leq 3.0$  contractions/

min; 21 and 53% respectively) (Fanchin *et al.*, 1998). This may result from the mechanical expulsion of embryos from the uterine cavity.

In addition, more recent work on the hormonal regulation of uterine contractility led us to speculate that utero-relaxation mediated by progesterone during the luteal phase takes a longer time to be fully established in ovarian stimulation compared with the menstrual cycle (Fanchin *et al.*, 2000a). This may constitute a plausible explanation for the high uterine contraction frequency seen at the time of non-cavitating embryo transfer in ovarian stimulation.

Based on this documentation, we decided to investigate the possible advantages of delaying embryo transfer so that embryos reach the uterine cavity when contractility is attenuated and conditions for embryo implantation are improved. For this, we compared uterine contraction characteristics on the day of HCG administration, the day of non-cavitating embryo transfer (HCG + 4), and the day of blastocyst transfers (HCG + 7).

## Materials and methods

### Patient characteristics

We prospectively studied 43 consecutive ovarian stimulation cycles for IVF-embryo transfer undertaken in 43 infertile women. Only women who were  $\leq 38$  years of age, whose uteri were morphologically normal as confirmed by hysteroscopy and ultrasound scans, and who had at least two good quality non-cavitating embryos (defined as uniform sized and shaped blastomeres, ooplasm having no granularity and a maximum fragmentation of 10%) available for embryo transfer were included. Clinical indications for IVF-embryo transfer were sperm abnormalities (63%), tubal abnormalities (21%), unexplained infertility (14%), and endometriosis (2%). An informed consent was obtained from all patients and this investigation received the approval of our internal institutional review board.

### Ovarian stimulation protocol

A single injection of a time-release gonadotrophin releasing-hormone (GnRH) agonist, triptorelin, (3.0 mg i.m., Decapeptyl®; Ibsen-Biotech Laboratories, Paris, France) was administered on cycle day 2. Eighteen days later, complete pituitary desensitization was confirmed by documenting low plasma oestradiol  $< 40$  pg/ml and luteinizing hormone (LH)  $\leq 2$  mIU/ml concentrations. Patients also had a conventional ultrasound examination to exclude ovarian cysts and verify that endometrial thickness was  $< 5$  mm. Recombinant FSH therapy (Puregon®; Organon Pharmaceuticals, Saint-Denis, France) was then initiated at a dosage of 225 IU/day for the first 5 days of ovarian stimulation. Further FSH doses and timing of HCG (Gonadotrophine Chorionique Endo®; Organon Pharmaceuticals, 10 000 IU, i.m.) administration were adjusted according to the usual criteria of follicular maturation determined by ultrasound and oestradiol findings. Administration of HCG was performed when at least three follicles exceeded 17 mm in diameter and oestradiol concentrations per mature follicle ( $\geq 17$  mm in diameter) were  $> 300$  pg/ml. Oocytes were retrieved 36 h after HCG administration by transvaginal ultrasound-guided aspiration.

All embryo transfers were performed 2 days after oocyte retrieval using Frydman catheters (CCD Laboratories, Paris, France). Luteal phase was supported with progesterone (Crinone® 8%; Ares-Serono SA, Geneva, Switzerland) administered daily by vaginal route starting on the evening of embryo transfer.

### Uterine contractility and hormonal assessment

All women underwent three sequential ultrasonographic and hormonal evaluations that took place on the day of HCG administration, just before the actual non-cavitating embryo transfer (4 days after HCG, HCG + 4), and on the day when blastocysts are commonly obtained and transferred (HCG + 7). Two minute ultrasound scans of a sagittal plane of the uterus were performed using a 7.5 MHz transvaginal probe (Siemens Elegra®; Siemens SAS, Saint-Denis, France) at approximately 1100 h by one single operator. Environmental conditions were standardized throughout ultrasound examinations. The present study respected similar methodological characteristics as previously described (Fanchin *et al.*, 1998, 2000a).

Briefly, images were digitized on-line using a two image/s rate with a computer-assisted image analysis system (I6TEC 3.1.2®; I6DP, Paris, France). As represented in Figure 1, uterine contraction frequency was assessed on time-mode graphs generated electronically using three-dimension reconstruction software (I6TEC 3.1.2®; I6DP). For this, instead of swapping the ultrasound probe as for volume acquisition and three-dimension reconstruction, the probe was kept steady and two-dimension images were acquired over time for 2 min. Hence, in the electronic matrix, the *z* axis, instead of being the third dimension of volume, was represented by time. In the time-mode graphs, uterine contraction frequency was identified by the number vertical displacements of the myometrial-endometrial interface and of the uterine cavity line over time. Precision of uterine contraction frequency measurements, expressed as interassay coefficient of variation, was 8%.

Direction of uterine contractions was assessed visually according to a 20 image/s rate (10 times the normal speed) and classified arbitrarily into four types: cervix-to-fundus or retrograde, fundus-to-cervix or antegrade, antagonistic (contractions starting simultaneously on the cervix and on the fundus and meeting on the middle of the uterus) and non-propagated contractions (local myometrial activity).

Serum FSH was measured by an immunometric technique using an Amerlite® kit (Ortho Clinical Diagnostics, Strasbourg, France). Intra-assay and interassay CV were, respectively, 5 and 7% and sensitivity was 0.1 mIU/ml for FSH. Serum progesterone was measured by radioimmunoassay using a I<sup>125</sup> Progesterone Coatria® kit (Bio-Mérieux, Paris, France). Sensitivity was 0.05 ng/ml and intra-assay and interassay CV were, respectively, 8 and 11% for progesterone. Serum oestradiol was determined by an immunometric technique using an Estradiol-60 Amerlite® kit (Ortho Clinical Diagnostics). Sensitivity was 14 pg/ml, and intra-assay and interassay CV were 8 and 9% for oestradiol respectively.

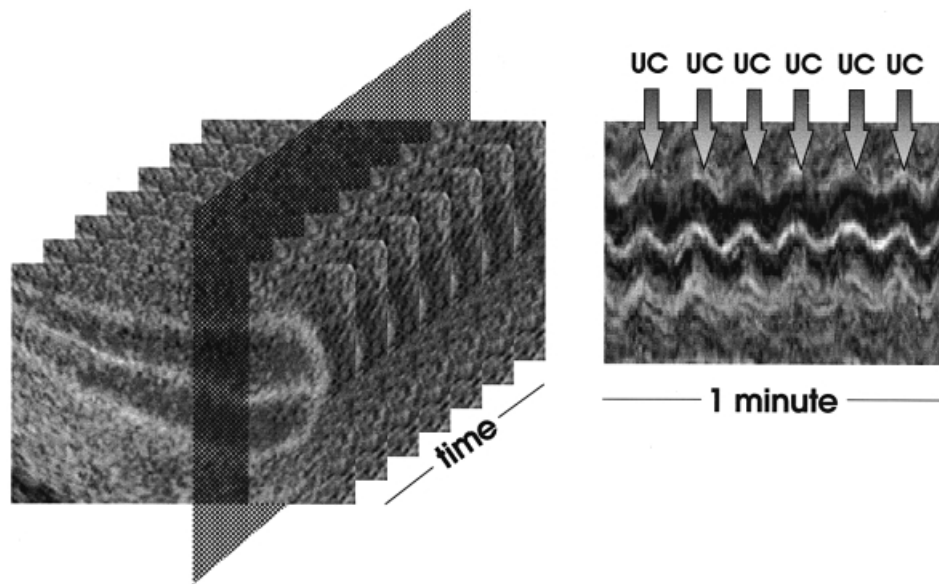
### Statistics

Measures of central tendency used were means and measures of variability were standard errors. When data distribution was non-parametric, medians and ranges were used. Changes in uterine contraction frequency and ovarian hormone levels were assessed using the paired Student's *t*-test and repeated-measures analysis of variance (ANOVA) when appropriate. Hormonal influence on uterine contractility was assessed using simple regression. A *P* value of  $< 0.05$  was considered statistically significant.

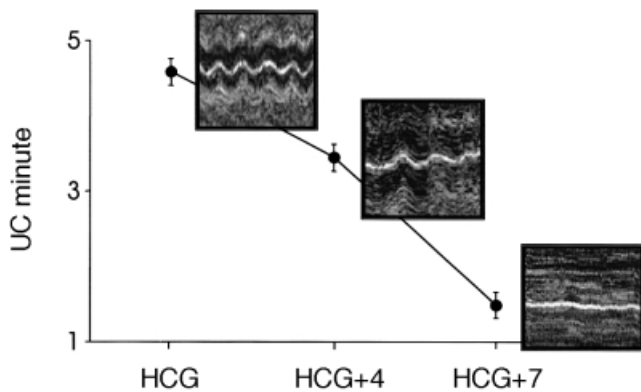
## Results

### Patients, ovarian stimulation and embryology data

Median age of patients was 33.0 years (range: 26–38), and mean cycle day 3 serum FSH and oestradiol concentrations, measured in a prior cycle, were within the normal range at  $5.1 \pm 0.2$  mIU/ml and  $27 \pm 2$  pg/ml respectively. Ovarian



**Figure 1.** Computerized assessment of uterine contraction frequency. After images of a sagittal plane of the uterus were acquired over time at a rate of two images/sec (left panel), time-dependent changes in myometrial-endometrial interfaces that correspond to uterine contractions (UC) were assessed (right panel). Vertical arrows indicate uterine contractions.



**Figure 2.** Progressive decrease of uterine contraction frequency from the day of HCG administration to HCG + 7 (expected day of blastocyst transfers) ( $P < 0.001$ , repeated-measures analysis of variance).

stimulation lasted  $11.3 \pm 0.2$  days and required  $32.4 \pm 1.7$  75 IU FSH ampoules. On the day of HCG administration, serum progesterone and oestradiol concentrations reached  $0.81 \pm 0.12$  ng/ml and  $2496 \pm 134$  pg/ml respectively. Mean numbers of mature oocytes retrieved and available embryos were  $10.6 \pm 0.6$  and  $5.6 \pm 0.4$  respectively. Median number of embryos transferred was three (range: 2–4).

**Uterine contractility**

Changes in uterine contraction (UC) frequency from the day of HCG administration to HCG + 7 are depicted in Figure 2. Mean UC frequency, which reached  $4.4 \pm 0.2$  UC/min (range: 1.5–8.5) on the day of HCG administration, decreased slightly yet significantly on HCG + 4 at  $3.5 \pm 0.2$  UC/min (range: 1.0–7.5) ( $P < 0.003$ ). On HCG + 7, an additional and noticeable reduction of UC frequency was observed ( $1.5 \pm 0.2$  UC/min, range: 0.0–3.5,  $P < 0.001$ ). The prevalence of each uterine contraction type (cervix-to-fundus or retrograde, fundus-to-cervix or antegrade, antagonistic, and non-propagated) was 57,

32, 9, and 2% respectively on the day of HCG administration. This frequency distribution remained comparable on HCG + 4 (56, 25, 14, and 5% respectively). On HCG + 7, however, prevalence of uterine contraction changed as compared with HCG administration (31, 23, 23, and 25% respectively), with a reduction of retrograde uterine contraction ( $P < 0.01$ ) and a more pronounced increase in non-propagated UC ( $P < 0.001$ ).

As expected, mean serum progesterone concentrations increased after HCG administration from  $0.72 \pm 0.07$  ng/ml to  $85.2 \pm 6.9$  ng/ml on HCG + 4 ( $P < 0.001$ ). A further increase in progesterone concentrations occurred from HCG + 4 to HCG + 7 ( $134.5 \pm 11.1$  ng/ml,  $P < 0.001$ ). Serum oestradiol concentrations fell after HCG administration from  $2497 \pm 134$  pg/ml to  $1058 \pm 61$  pg/ml on HCG + 4 ( $P < 0.001$ ), before increasing on HCG + 7 ( $1952 \pm 81$  pg/ml,  $P < 0.001$ ).

On the day of HCG administration, serum progesterone concentrations failed to correlate with uterine contraction frequency. On HCG + 4, as expected, we observed a negative and significant correlation between serum progesterone concentrations and UC frequency ( $r = -0.43$ ;  $P < 0.004$ ). Similarly, on HCG + 7, serum progesterone concentrations and UC frequency correlated negatively ( $r = -0.34$ ;  $P < 0.03$ ). Serum oestradiol concentrations did not correlate with UC frequency at any time. No statistically significant correlation was identified between direction of uterine contraction and serum progesterone or oestradiol concentrations.

**Discussion**

The present study was conducted to investigate the changes in uterine contractility that occur during the first 7 days following HCG administration, i.e. up to the time when blastocysts are commonly transferred into the uterus. It was prompted by the reported relationship between uterine contractions and embryo implantation (Ijland *et al.*, 1997, 1998), and

by our previous findings that pregnancy and implantation rates are decreased when contractile frequency is exacerbated on the day of non-cavitating embryo transfer (Fanchin *et al.*, 1998). The present results indicate a remarkable fall in uterine contraction frequency on HCG + 7. This may account, at least in part, for the putative increase in implantation potential of blastocysts. Furthermore, we observed a significant re-organization of uterine contraction direction.

Because the number of blastocyst transfers is limited at our institution, it was impractical to enrol enough cases to assess uterine contraction characteristics on the day of actual blastocyst transfers. In an effort to overcome this limitation, we studied a selected population (women aged  $\leq 38$  years, displaying adequate ovarian follicular reserve and with at least two good quality embryos available for embryo transfer on HCG + 4) who had good probability of reaching the blastocyst transfer. Therefore, these methodological characteristics may authorize the extrapolation of present uterine contraction data observed on HCG + 7 (theoretical day of blastocyst transfers) to real blastocyst transfers conditions. However, it is noteworthy that possible direct and local influence of embryos present in the uterine cavity on HCG + 4 and uterine contractility on HCG + 7 could not be ruled out by the present study and need further investigation.

The observed reduction of uterine contraction frequency after HCG administration presumably results from the utero-relaxing properties of progesterone, both secreted by multiple corpora lutea and administered exogenously after embryo transfer. In support of this, the present data indicate that serum progesterone concentrations correlate negatively with uterine contraction frequency not only on HCG + 4, thus confirming our earlier reports (Fanchin *et al.*, 1998, 2000a), but also on HCG + 7. Conversely, the myo-relaxing action of progesterone may depend on both its absolute circulating levels and the duration of uterine exposure to progesterone. In agreement with this hypothesis, uterine contraction frequency decreases progressively during the luteal phase of the menstrual cycle (Abramowicz and Archer, 1990; Lyons *et al.*, 1991). The luteal reduction of uterine contractile activity potentially assists the embryo implantation process. Accordingly, in natural conditions, the entry of the morula into the uterine cavity does not occur before ~72–96 h after fertilization (Croxtatto *et al.*, 1978), when uterine activity is already reduced.

Hence, it is conceivable that 2- to 8-cell embryo transfers are often performed too early when utero-relaxation mediated by progesterone has not been fully exerted. Indeed, despite serum progesterone reaching very high mean concentrations on HCG + 4 ( $>85$  ng/ml), the duration of uterine exposure to this hormone at that time remains relatively short ( $<3$  days) (Fanchin *et al.*, 2000b). This brief exposure to progesterone, together with the possibility of persistent utero-stimulating effects of supra-physiological oestradiol concentrations from ovarian stimulation (Fanchin *et al.*, 2000a), may contribute to the insufficient utero-relaxation observed on the day of non-cavitating embryo transfers (HCG + 4). On HCG + 7, however, duration of uterine exposure to progesterone is much longer and oestradiol-induced utero-excitability may be decreased, which concur to explain utero-relaxation.

Further, the present study showed an increasing prevalence of non-propagated uterine contractions from the day of HCG administration to HCG + 7. Whether or not this phenomenon may be attributed to an action of progesterone as well as its possible role on embryo implantation deserves further elucidation. Finally, it is important to mention that the contractile activity of the uterus is a complex phenomenon that involves either superficial or profound muscle layers with generalized and local consequences. Hence, the complete assessment of all uterine contractility characteristics by ultrasound may sometimes be difficult. This methodological limitation led us to consider only contraction frequency and, to a lesser extent, direction of propagation in our present and past ultrasound studies (Fanchin *et al.*, 1998, 2000a). Indeed, recent data indicated that, during the luteal phase, the uterine fundus shows only relative quiescence and the residual contractile may be important to blastocyst positioning (Kunz *et al.*, 2000).

In conclusion, concurring with the putative embryo selection through extended culture, the nearly quiescent status of the uterus reached on the day of blastocyst transfers may avoid embryo displacement in the endometrial cavity and, therefore, assist implantation. Hence, the present data may offer an additional explanation of the high implantation rates reported after blastocyst transfers (Gardner *et al.*, 1998a,b; Ménézo *et al.*, 1998; Schoolcraft *et al.*, 1999; Huisman *et al.*, 2000). Further, based on these results, extending culture and transferring blastocysts instead on 2- to 8-cell embryos may be opportune in cases of high uterine contraction frequency ( $\geq 5$  contractions/min) (Fanchin *et al.*, 1998) on the day of non-cavitating embryo transfers.

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