RBMOnline - Vol 14. No 6. 2007 686-692 Reproductive BioMedicine Online; www.rbmonline.com/Article/2803 on web 26 April 2007

Article

Embryo transfer and luteal support in natural cycles

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Abstract

Embryo transfer policy and luteal supplementation was reviewed, comparing literature data and the results from the Maribor IVF Centre. A retrospective analysis of 1024 cycles in patients undergoing IVF, intracytoplasmic sperm injection (ICSI) or testicular sperm aspiration in unstimulated cycles was carried out using four different approaches for cycle monitoring. This showed that the most successful protocol for monitoring was administration of human chorionic gonadotrophin (HCG) when serum oestradiol was >0.49 nmol/l and follicle diameter was at least 15 mm. The implantation rate per transferred embryo was higher when a blastocyst was transferred (42.8%) rather than a day-2 embryo (23.5%) in the same monitoring protocol. Analysis of the influence of patient age on the success of oocyte retrieval, oocyte fertilization, embryo transfer rate and delivery rate demonstrates that patient age does not influence the rate of positive oocyte retrieval or fertilization rate as much as it influences pregnancy rate per embryo transfer. The delivery rate per cycle was dramatically influenced by age in patients over 38 years. There is no clear evidence in the literature as to whether luteal phase support is necessary in natural cycles for IVF/ICSI. Comparing the data, a higher pregnancy rate was observed if HCG was administered after embryo transfer.

Keywords: *age dependence, delivery rate, IVF, natural cycle, pregnancy rate*

Introduction

The aim of this paper is to compare different monitoring protocols, embryo transfer policies and luteal support results coming from a single centre reporting experience with over 1000 natural IVF/intracytoplasmic sperm injection (ICSI) cycles. The first IVF attempts involved oocyte collection in natural menstrual cycles. After the first successfully achieved IVF pregnancies in natural cycles (Edwards *et al.*, 1980b) and use of the technique in the 1980s, the procedure was replaced in the following decade by the more successful oocyte retrieval in ovarian stimulation cycles. During the past 30 years, ovarian stimulation protocols with recruitment of multiple follicles containing fertilizable oocytes for IVF have been constantly evolving, with the aim of improving the success of IVF centres and the procedure itself.

Even after an early optimistic announcement of the revival of IVF in natural cycles at the end of the 1980s (Garcia, 1989), such procedures were limited to very few centres. The proportion of IVF in natural cycles in worldwide statistics is not known. In the European IVF Registry (EIM), natural cycles are not reported separately (Andersen *et al.*, 2006). Compared with IVF in stimulated cycles, pregnancy rates are lower in unstimulated cycles (Daya *et al.*, 1995). IVF in unstimulated cycles can never reach figures that exceed the fecundity rate of fertile couples. The opportunity to transfer more embryos at the same time, which allows high pregnancy rates, made ovarian stimulation the method of choice in recent decades, despite the fact that multiple pregnancy rates usually exceed 25% (Andersen *et al.*, 2006). The reason for revival of IVF in natural cycles is the new definition of IVF success, which differs from the one in the past (number of oocytes and embryos, implantation or pregnancy rates). The patients are better informed about the risks involved in IVF, and they wish for healthy singleton babies only. If one embryo only needs to be transferred, why stimulate the cycle? With one oocyte available the chances of success might be reduced, but that would be compensated by the flexibility of doing more IVF cycles per patient in a given year (Garcia, 1989). IVF in natural cycles offers a successful outcome for many of these expectations. The method is simple and patient-friendly, cost and patient discomfort during the procedure are low, and the treatment is readily accessible. The benefit of IVF natural cycles is more evident in women under 35 years of age. In those over 35, the benefits of IVF in the natural cycle are less evident and the opportunity to transfer multiple embryos in these patients seems to be advantageous (Phillips *et al*., 2007).

In recent literature, the attention is again focused on such an approach. In completely natural or unstimulated cycles, similar results in IVF and ICSI and testicular sperm aspiration (TESA) patients were reported (Vlaisavljevic *et al*., 2001b). These first results of ICSI/TESA in natural cycles were followed by others in ICSI patients (Lukassen *et al*., 2003), or ICSI with frozen–thawed percutaneous epididymal sperm aspiration-retrieved spermatozoa (Kadoch *et al*., 2005).

At the same time, more attention was given to 'simplified' or 'minimal' stimulation protocols (Mausher *et al*., 2006). Part of such an approach is a modification of the natural cycle consisting of the administration of mild stimulation with recombinant FSH (rFSH) and gonadotrophin-releasing hormone (GnRH) antagonist in the late follicular phase (Rongieres-Bertrand *et al*., 1999; Pelinck *et al*., 2005, 2006).

Selection of patients

Only patients no older than 45 years with ovulatory cycles were included. Indications for IVF and ICSI (including TESA) in the authors' centre were the same as in stimulated cycles. Indications for assisted reproduction in non-stimulated (natural) cycles were: substitute for stimulated cycle, management of poor responders to ovarian stimulation, natural cycles for testing of oocyte ability for fertilization, and embryo development compared with oocytes coming from previous unsuccessful stimulated cycles (Vlaisavljevic *et al*., 2001a). Changes in public expectations regarding optimum IVF treatment re-introduced a more natural approach to IVF, including minimum stimulation protocols and in-vitro maturation (IVM) (Edwards, 2007). The IVM procedure is attractive for replacing stimulation in polycystic ovarian syndrome patients, where oocyte retrieval can be performed even though the follicles are not bigger than 10–12 mm (Vlaisavljevic *et al*., 2006). IVM and IVF were not used simultaneously in natural cycles, as was reported by Chian *et al*. (2004).

Following public expectation for a more patient-friendly IVF procedure, single embryo transfer, avoiding multiple pregnancies and hyperstimulation syndrome, information about the opportunity to replace one stimulated cycle with four natural cycles at the beginning of treatment with IVF/ICSI was given to those patients whose insurance covers the total IVF cost (medication and treatment). This approach was accepted by Slovenian IVF centres.

Criteria for human chorionic gonadotrophin administration

Some centres reported their results in natural cycles where LH blood monitoring was used for the timing of oocyte retrieval. The disadvantage of such an approach was need to check serum LH several times daily and the unpredictable time of oocyte retrieval (Lenton *et al*., 1992). For that reason, such an approach is generally avoided.

An untimely LH surge is the most common problem in monitoring natural cycles. Monitoring of follicle development by ultrasound only is associated with high cancellation rates due to premature ovulation. Cancellation frequency varies between 24.0 (Bauman *et al*., 2002) and 40–50% when ultrasound was the only monitoring tool (Aboulghar *et al*., 1995; Vlaisavljevic *et al*., 1995). A systematic review of recent literature shows there are no strict criteria for deciding on the correct moment to induce final oocyte maturation with human chorionic gonadotrophin (HCG) administration (Pelinck *et al*., 2002). The criteria are not only arbitrary, but they are flexible. The data from the literature suggest the administration of HCG when the dominant follicle measures >16–20 mm or oestradiol concentrations were indicating a satisfactory follicular development (0.7 to >1.1 nmol/l), but the cancellation rate was still unacceptably high, at between 20 and 30%.

To make the criteria more strict, the administration of HCG at a smaller follicle diameter is suggested, to diminish the cancellation rate caused by the LH surge or spontaneous ovulation and to make the decision uniform for all patients (Reljic and Vlaisavljevic, 1999; Vlaisavljevic *et al*., 2001b).

Ultrasound assessment, carried out to ensure that HCG was administered on the first day when the dominant follicle reached a size >15 mm, and oestradiol concentrations >0.49 nmol/l indicated satisfactory follicular development. Cancellation rates and the number of unsuccessful oocyte retrievals were 9.7% in IVF and 9.3% in ICSI natural cycles (Vlaisavljevic *et al*., 2002). However, in predicting the outcome of natural IVF/ICSI cycles, the importance lies not in the oestradiol concentration on the day of HCG administration, but in the oestradiol ratio between values measured 12 h before and 12 h after HCG administration (Reljic *et al*., 2001).

Embryo transfer policy

The results of 1800 IVF natural cycles in a systematic review by Pelinck *et al*. (2002) give an embryo transfer rate of 45.5%, a pregnancy rate per cycle of 7.2% and a pregnancy rate per transfer of 15.8%. No delivery rate was reported.

A retrospective analysis of 1024 oocyte retrievals in natural IVF/ICSI/TESA cycles from the Maribor centre showed that 71 babies were delivered (one set of monozygotic twins). The embryo transfer rate per oocyte retrieval was 46.9%, clinical pregnancy rate per ET 19.8%, pregnancy rate per oocyte retrieval 9.3% and delivery rate per oocyte retrieval 6.9%. Therefore, 6.8 embryos needed to be transferred per baby born. Maternal age was an important factor in determining the success rate of oocyte retrieval and embryo transfer as well as the outcome of IVF/ICSI. **Figures 2–4** show the results and analyse the relationship between maternal age, pregnancy and delivery in natural cycles established at Maribor Teaching Hospital.

The exact values of natural human fecundity are difficult to estimate. The data required for such estimation are the distribution of the interval from marriage to the first birth or from the resumption of conception risk after contraception to the subsequent birth (Bongaarts, 1975). Using such an approach, the author found that fecundity mean values in the observed populations ranged from 0.18 to 0.31. These values should be taken into account when assessing the result expected from the IVF procedure in one natural cycle.

Blastocyst transfers lead to a disappointingly low number of embryo transfers per aspiration (29.4%) versus 51.5% if a day- 2 embryo is transferred. Implantation rates per embryo transfer were higher in blastocyst transfers (42.8%) leading to the same pregnancy rate per cycle as if an embryo was transferred on day 2 after oocyte retrieval (23.5%). The expected pregnancy rate calculated per embryo available on day 2 (15.4 versus 14.3%) was similar in both groups, and it was not affected by oocyte culture to the blastocyst stage (Vlaisavljevic *et al*., 2001a).

Embryo transfer of a blastocyst coming from a natural cycle is an option for patients who prefer embryo self-selection ('not to have the transfer'), in contrast to those who 'want to have the transfer', although a poor quality embryo may be transferred on day 2 or 3. Endometrial thickness measurement has established its place in IVF procedure monitoring, but the issue of its importance in predicting cycle outcome remains controversial. The distribution of thickness and echographic patterns of the endometrium is similar in spontaneous and stimulated cycles. Several studies (Fleisher et al., 1986; Wolman et al., 1994) suggest that the best results are achieved if endometrial thickness is 8–9 mm or more or when it appears to be 'trilaminar', and poor results if the thickness is <8 mm, or if it appears homogenous on the day of HCG administration. Prospectively collected data on endometrial thickness, endometrial pattern and subendometrial activity were analysed by Vlaisavljevic et al. (2001c). Pregnant women tended to have a thicker endometrium on day +12 after oocyte retrieval than non-pregnant women. There appears to be no appreciable difference between the endometrial movement and thickness in pregnant and non-pregnant women. In combining three echographic variables (thickness, pattern and movement) and hormone concentrations (oestradiol and progesterone), only progesterone on day +12 could be used to predict the IVF outcome. It was concluded that subendometrial contractility plays no important role in implantation after embryo transfer. Based on ultrasound characteristics such as subendometrial movement, thickness and pattern on the day of embryo transfer in the natural IVF/ICSI cycle have no predictive value for embryo implantation (Kuder et al., 2002).

Luteal support

Since the early days of IVF treatment, it has been presumed that luteal phase abnormality in stimulated cycles is caused by elevated concentrations of oestradiol due to ovarian stimulation (Edwards *et al*., 1980a). Follicle aspiration and removal of the oocyte–cumulus complex together with large quantities of granulosa cells and layers (which is the most important progesterone secreting unit in the subsequent corpus luteum) was recognized as a possible mechanism of luteal insufficiency (Garcia *et al*., 1981). This was not recognized as an important mechanism in stimulated cycles (Fauser and Devroey, 2003). Under these circumstances, it was mutually accepted to either supplement the luteal phase by progesterone or rescue the corpus luteum by the administration of HCG. The need for luteal support after embryo transfer coming from a natural cycle is still not definitely evaluated. There are no randomized trials or meta-analyses on this subject (Pritts and Atwood, 2002), as is the case in stimulated IVF or ICSI cycles, in co-treatment with GnRH agonist or antagonist (Daya and Gunby, 2004). It is not clear whether luteal phase support is necessary in natural cycles.

A review of the literature related to natural IVF cycles does not give a definitive answer (Pelinck *et al*., 2002). These authors found 10 reports where luteal support was part of the procedure and it was given after 535 embryo transfers, and only two reports where luteal support was not given in 70 reported embryo transfers. In eight studies, luteal support was not mentioned.

From the articles on corpus luteal function after follicle aspiration for oocyte retrieval for IVF in the laparoscopic era, there is conflicting evidence as to whether follicular aspiration in a spontaneous cycle leads to a defective luteal phase.

Garcia *et al*. (1981) report a grossly defective luteal phase in a group of 32 patients after oocyte retrieval in spontaneous cycles, especially among those in whom repeated aspiration was performed. There was a statistically significant decrease in the amount of progesterone in aspirated cycles involving most vigorous aspirations and the greatest number of follicle washes were performed, as compared with the unaspirated control cycles. Oestradiol values follow similar characteristics. The same authors analysed the number of viable granulosa cells aspirated. The average number of viable granulosa cells aspirated from follicles measuring at least 18 mm in diameter was 4.72×10^6 (minimum of 0.2×10^6 to maximum of 12×10^6). The number of active granulosa cells in the mature follicle has been estimated to be approximately 50×10^6. The number of removed granulosa cells after the first aspiration (approximately 10%) and repeated aspirations may play some role in the function of the corpus luteum (Garcia *et al*., 1981).

Frydman *et al*. (1982) observed only a transitory deficiency in plasma progesterone after the aspiration of a spontaneous pre-ovulatory follicle. They studied 20 patients in two successive cycles. Following follicle puncture, the progesterone concentration dropped significantly on day 3 ($P < 0.01$) and returned to normal on days 6 and 9. The duration of the luteal phase was not modified by follicle puncture.

Contrary to these two reports, Kerin *et al*. (1981) reported a normal luteal phase after follicle aspiration and oocyte recovery in spontaneous cycles. No significant differences in any of the parameters measured throughout the menstrual cycle (oestradiol, 17-OH progesterone and progesterone) were found between the women who had their pre-ovulatory follicle aspirated and the control group of women who had ovulated spontaneously. The apparent reduction in the mid-luteal oestradiol concentration in women who had their follicle

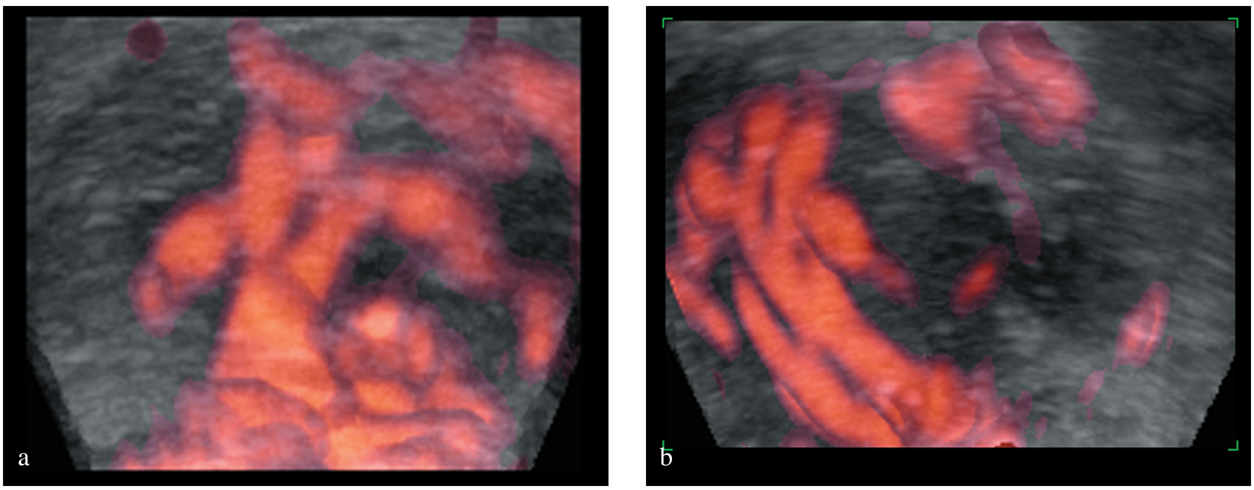

Figure 1. (**a**), (**b**) 3D power Doppler visualization of the vascular network developing after spontaneous follicle rupture in a natural cycle.

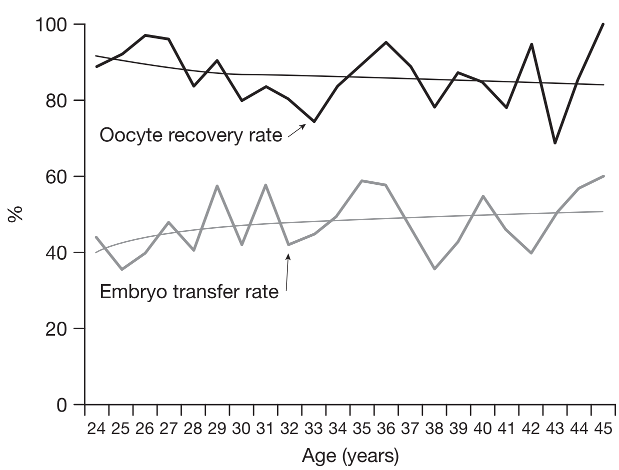

Figure 2. Positive oocyte retrieval rate and embryo transfer rate per oocyte retrieval in 1024 natural cycles for IVF/ intracytoplasmic sperm injection (Maribor IVF).

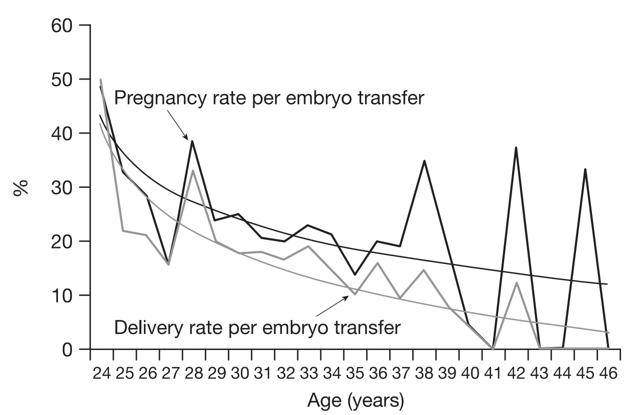

Figure 3. Pregnancy rate and delivery rate per embryo transfer in 480 embryos coming from natural cycles for IVF/ intracytoplasmic sperm injection (Maribor IVF).

aspirated did not achieve statistical difference compared with the control group. The maximum difference occurred on day 5 after oocyte retrieval. It should be kept in mind that in all studies, the pre-ovulatory follicle grew to a diameter of at least 18 mm (maximum 23 mm) and the pre-ovulatory peripheral blood oestradiol concentration was greater than 1.1 nmol/l. These conditions are perhaps optimal for forming an adequate corpus luteum. Dynamic changes in the form of a 3- to 4-fold increase in progesterone concentration in peripheral blood during the 30 h before ovulation, functional luteinization of the pre-ovulatory follicle and a significant fall in oestradiol concentration occurring in the 16 h prior to ovulation can be important for initiating maturational changes in enzymatic and steroid function and programming of the pre-ovulatory follicle to behave as luteal tissue (Kerin *et al.*, 1981). This is not the case in all non-stimulated cycles for IVF that are triggered with HCG at different stages of follicular growth. Therefore, some different approaches to luteal supplementation need to be discussed.

Feichtinger *et al.* (1982) found a luteal phase deficiency in only two patients from among 32 non-stimulated cycles after laparoscopic oocyte recovery. Both patients exhibited a transitory drop in progesterone on day 7 after oocyte retrieval, and luteal support with dydrogesterone was given prophylactically.

Perifollicular vascularization and corpus luteum formation

Different Doppler modalities were used in recognizing 'pregnancy-quality' follicles in unstimulated cycles by assessing perifollicular blood flow. Gavric-Lovrec *et al.* (2001) analysed quantitative characteristics of perifollicular blood flow in the pre-ovulatory period in 210 unstimulated cycles. Measurements of resistance index, pulsatility index and peak systolic velocity were carried out on the day of HCG administration, on the following day and the day of oocyte retrieval. Quantitative pulsed Doppler indices are not useful in recognizing pregnancy-quality follicles. The perifollicular space of follicles associated with conception includes a high percentage of tissue showing a power Doppler flow signal. It can be postulated that the follicles containing oocytes able to produce a pregnancy have a distinctive and more uniform perifollicular vascular network (Vlaisavljevic *et al.*, 2003). This network is not only an important part of the perifollicular space on the day of oocyte retrieval, but very active in proliferation of new vessels on the days following HCG administration.

Aspiration of the follicle, irrigation with flushing media and re-aspiration of the follicle results in damage to the fine vascular network of blood vessels (**Figure 1**). Damage to this theca interna vascular network produces a bloodstained aspirate followed by the reaccumulation of blood within the collapsed aspirated follicle in the next few minutes. This is a frequent observation after the 'curettage' of the inner granulosa cell layer during the aspiration procedure.

There is good experimental evidence that high oestradiol concentrations in the pre-ovulatory follicle correspond with the number of granulosa cells in follicular fluid. There is evidence that following the LH surge, human granulosa cell mitotic activity is inhibited, indicating that there is probably no further increase in granulosa cell number following ovulation (McNatty and Sawers, 1975). The quantity of aspirated granulosa cells during oocyte retrieval from non-stimulated follicles depends on their diameter. In follicles >18 mm in diameter, the quantity of viable granulosa cells in the aspirate is double that found in the aspirate of follicles <17 mm in diameter (Garcia *et al.*, 1981).

The administration of 5000 IU HCG to trigger final oocyte maturation in dominant follicles influences corpus luteum function on subsequent days. Only HCG, and not endogenous

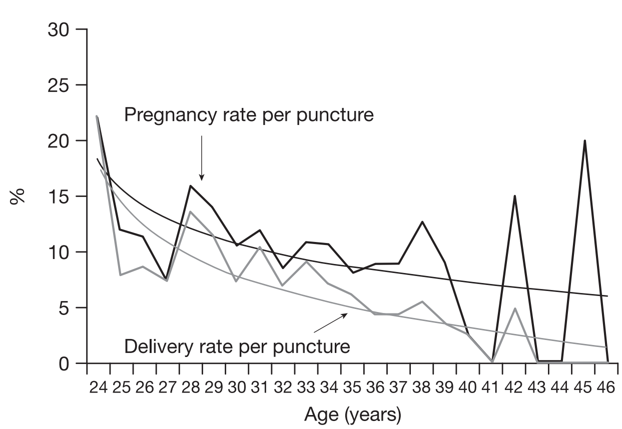

Figure 4. Pregnancy rate and delivery rate per oocyte retrieval in 1024 natural cycles for IVF/intracytoplasmic sperm injection (Maribor IVF).

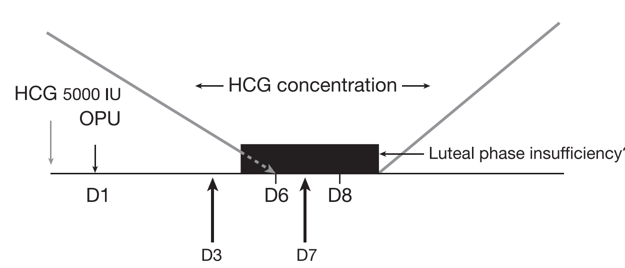

Figure 5. Luteal phase insufficiency after IVF in a natural cycle. Luteal support is given to cover the 'gap' when the exogenous human chorionic gonadotrophin (HCG) support disappears and the time when endogenous HCG from early pregnancy starts to rise after transfer.

LH, has the capacity to prolong corpus luteum half-life. The dose necessary to support the corpus luteum has not been defined.

The natural developing follicles contain more HCG-stained granulosa cells than the stimulated ones (Gersak and Tomazevic, 1996). Their ability to secrete progesterone depends on the day of the luteal phase. Progesterone secretion 3 h after HCG administration to 'in-vitro' corpora lutea is significantly higher in the middle luteal phase than in the early and late luteal phase (Vega *et al*., 1987). Progesterone concentrations depend on the pre-ovulatory bolus of HCG and become very low in the mid-luteal phase in ovarian stimulation (Beckers *et al*., 2000, 2003). In natural cycles significantly lower serum progesterone was noted following oocyte retrieval (Frydman *et al*., 1982; Mahmood and Templeton, 1991). Although all results were within the normal range seen in the control cycles, it is not known whether a biological significance should be attributed to the minimum differences in observed progesterone concentrations after oocyte retrieval. Luteal support is given to cover the 'gap' when the exogenous HCG support disappears (day 5–6) and the time when endogenous HCG resulting from early pregnancy starts to rise (day 9–12) after transfer (**Figure 5**).

Generally, luteal supplementation in natural IVF cycles is neither universal nor evidence based. In the literature, supplementation with vaginal or intramuscular administration of progesterone, oral administration of dydrogesterone, or a single 'booster' dose of HCG can be found more frequently than cases where luteal supplementation is completely omitted.

A retrospective analysis of different luteal phase supplementation in natural cycles was performed in 256 embryo transfers. In the group where dydrogesterone (Dabroston 30 mg; Duphare) was used, 96 embryos were transferred, 12 (12.5%) implanted and eight (8.3%) resulted in delivery. In the group where the luteal phase was supplemented with HCG (1500 IU on day 3 and day 7; Pregnyl; Organon), 158 embryos were transferred, 37 (23.4%) implanted and 25 (15.8%) resulted in delivery (Vlaisavljevic *et al*., 2001b). This difference in implantation rate was suggestive but not significant (95% CI 5.7–19.3 versus 95% CI 16.7–30.2).

Conclusions

Natural cycles offer a simpler, faster and less invasive form of IVF and ICSI than the more common conventional stimulated cycles. Unstimulated cycles monitored with ultrasound and the combination of serum oestradiol and urinary LH can produce an acceptable pregnancy rate after IVF and ICSI. A lower cancellation rate was obtained when HCG was applied at lower values of serum oestradiol and smaller follicle diameter. A higher pregnancy rate was obtained if HCG was administered as luteal phase support after the embryo was transferred.

Improvements in laboratory techniques such as ICSI and blastocyst culture may increase the acceptability of natural cycles, giving this method a special position at the start of infertility treatment using assisted reproduction.

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Paper based on a contribution presented at the First World Congress on Natural Cycle/Minimal Stimulation IVF in London, UK, December 15–16, 2006.

Received 22 February 2007; refereed 7 March 2007; accepted 5 April 2007.