Similar morphokinetic patterns in embryos derived from obese and normoweight infertile women: a time-lapse study

J. Bellver*, A. Mifsud, N. Grau, L. Privitera, and M. Meseguer

Instituto Valenciano de Infertilidad, University of Valencia, Plaza de la Policía Local 3, Valencia 46015, Spain

*Correspondence address. Tel: +34-96-305-0900; Fax: +34-96-305-0999; E-mail: jose.bellver@ivi.es

Submitted on July 5, 2012; resubmitted on October 4, 2012; accepted on November 28, 2012

STUDY QUESTION: Does female obesity affect the dynamic parameters of embryo quality assessed by time-lapse analysis?

SUMMARY ANSWER: Female obesity does not affect the dynamic embryo quality as determined by image acquisition and time-lapse analysis.

WHAT IS KNOWN ALREADY: Female obesity impairs natural and assisted reproduction but there is no agreement on the specific contribution of gametes, embryos or endometrial receptivity. In this preliminary study the dynamic parameters of embryo quality are assessed for the first time by time-lapse analysis.

STUDY DESIGN, SIZE, DURATION: Two-year cohort retrospective study comparing embryos from three groups of patients according to the presence of infertility and/or obesity.

PARTICIPANTS AND SETTING: Participants attended a University-affiliated private clinic where ICSI was performed. Using an IVF incubator with a built-in camera designed to automatically acquire images at defined time points, we monitored individual embryos from 89 patients: 71 embryos from 13 obese infertile women, 242 embryos from 45 normoweight infertile women and 111 embryos from 31 normoweight fertile oocyte donors. The chronological pattern of cell divisions ( timings of cell cleavages) and other morphologic features (time-dependent cell size and nucleation) was recorded.

MAIN RESULTS AND THE ROLE OF CHANCE: Embryos from obese and normoweight infertile women showed similar cleavage patterns, but occurring more slowly, to those from fertile donors. These differences were statistically significant for t2 ( time of cleavage to two-blastomere embryo ) ( \( P = 0.016 \) ), t3 ( \( P = 0.014 \) ), t4 ( \( P = 0.003 \) ) and t5 ( \( P = 0.040 \) ).

LIMITATIONS, REASONS FOR CAUTION: These are preliminary data from a retrospective analysis with a limited sample size.

GENERALIZABILITY TO OTHER POPULATIONS: Not recommended until further studies using time-lapse analysis of a larger sample have been performed.

STUDY FUNDING/COMPETING INTEREST(S): None.

Key words: embryo / cell division / female obesity / blastocyst / time-lapse

Introduction

Obesity is a social, economic and health problem especially in developed countries where >30% of the adult population, both men and women, is affected (Flegal et al., 2010). The World Health Organization has suggested that by 2015 ~2.3 billion adults will be overweight and >700 million will be obese (WHO, 2006). Obesity is associated with several comorbidities and higher mortality rates (Kulie et al., 2011). From the point of view of reproduction, obese women suffer a significant increase in subfecundity, infertility and miscarriage rates, regardless of the presence of ovulatory disorders (Rich-Edwards et al., 2002; van der Steeg et al., 2008; Polotsky et al., 2010; Boots and Stephenson, 2011). The outcome is also impaired after using assisted reproduction techniques (ARTs). In fact, lower implantation...
and pregnancy rates, higher miscarriage rates and lower live birth rates have been shown in obese women following ART in a dose-dependent manner. Hence, the higher the BMI is, the lower the success rates are (Metwally et al., 2008; Bellver et al., 2010; Luke et al., 2011; Rittenberg et al., 2011).

The reduction in live birth rate is partly a result of the higher frequency of maternal and fetal complications during the second and third trimesters of pregnancy. However, an abnormal cross-talk between embryo and uterus has been suggested as the main reason for the impairment of implantation, pregnancy and ongoing pregnancy rates (Bellver et al., 2006).

Despite the fact that weight excess seems to be related to sperm anomalies in men (Martini et al., 2010) and to a reduction in oocyte competence, embryo quality and uterine receptiveness in women (van Swieten et al., 2005; Bellver et al., 2007; Metwally et al., 2007), there is as yet no consensus in the medical literature on the specific contribution of each of these components to the final poorer outcome. Concerning embryo quality, some studies have shown a negative effect of obesity through reduced fertilization rates, embryo cleavage rates, incidence of embryo transfer and number of embryos transferred or cryopreserved but other reports have found no such deleterious effects (reviewed by Bellver, 2008). Some authors have suggested that the manner in which obesity affects embryo quality cannot be ascertained by the static morphologic grading of embryos commonly employed in IVF laboratories (Bellver et al., 2010; Shah et al., 2011). Therefore, embryo morphology may not be a good predictor of success in the obese population.

Recently, time-lapse technology has been shown to improve embryo selection by adding new dynamic parameters of embryo quality in the IVF laboratory (reviewed by Montag et al., 2011; Basile and Meseguer 2012; Kirkegaard et al., 2012; Meseguer et al., 2012). The chronological pattern of cell divisions as well as of other morphologic features (cell size and nucleation) seems to determine the implantation potential of a specific embryo, leading to a hierarchical dynamic classification which helps in the selection of the most viable embryos for transfer (Cruz et al., 2011; Meseguer et al., 2011).

The aim of this preliminary study was to assess whether morphokinetic evaluation of embryos derived from obese women may be useful for defining new parameters of adverse embryo development in this population.

Materials and Methods

Study population

Eighty-nine women and 424 embryos assessed by time-lapse technology were retrospectively included in the present study between September 2009 and September 2011. Three groups were compared: 71 embryos from 13 obese infertile women (BMI: ≥30 kg/m²) in Group A; 242 embryos from 45 normoweight infertile women (BMI: 20–24.9 kg/m²) in Group B and 111 embryos from 31 normoweight fertile oocyte donors in Group C. In order to determine the influence of obesity and infertility on embryo dynamics, the inclusion criteria were as follows: time of infertility >12 months (Groups A and B), <38 years of age (Group C <35 years by Spanish law), no endocrine or autoimmune disease, normal uterus, presence of both ovaries, no endometriosis, no severe sperm alteration (>5 million motile sperm in the ejaculate) and no male partner (or recipient partner in Group C) obesity. In Groups A and B, tubal pathology, intrauterine insemination (IUI) failure (>4 failed previous attempts) or unknown infertility were the indications for IVF. Only first cycles of IVF were included.

The study was approved by the Institutional Research Board and Ethics Committee. All the included patients had previously given an informed consent for embryo morphokinetic evaluation.

Ovarian stimulation

No hormonal treatment (including gonadotrophins or oral contraception) was given in the 3 months preceding the beginning of the IVF cycle. Controlled ovarian stimulation (COS) was carried out with a long or short protocol. In the long protocol, the GnRH agonist leuprolide acetate (Procrin®; Abbott, Madrid, Spain) was employed for pituitary desensitization at a dosage of 0.25 mg; Merck-Serono, Madrid, Spain) was given daily from Day 6 of COS until the day of hCG administration. In the short protocol, a GnRH antagonist (Cetroide® 0.25 mg; Merck-Serono, Madrid, Spain) was given daily from Day 6 to COS until the day of hCG administration. Ovarian stimulation was performed using 150–300 IU of recombinant FSH (rFSH) (Gonal-F®; Merck-Serono, Madrid, Spain) or rFSH plus hMG (Menopur®; Ferring Pharmaceuticals, Madrid, Spain) daily, according to female age, basal hormone values, ovarian pattern at ultrasound and BMI. Gonadotrophins were administered from Day 3 of menstruation. Serial transvaginal ultrasound examinations and serum estradiol determinations were initiated on Day 5 of COS and repeated every 48 h to monitor the ovarian response. hCG (Ovitrelle®; Merck-Serono, Madrid, Spain) was administered s.c. when at least two leading follicles reached a mean diameter ≥18 mm. Transvaginal oocyte retrieval under ultrasound guidance was scheduled for 36 h later.

The protocol for endometrial preparation in oocyte recipients has been described elsewhere (Bellver et al., 2007). Two embryos were transferred in all cases on Day 3 of embryo development. Surplus good-quality embryos were cryopreserved by vitrification. Fourteen to 16 days after oocyte retrieval a serum βhCG level was measured, where a result >10 UI/L was considered positive. When βhCG was positive, a second βhCG test and a primary transvaginal ultrasound were performed 1 week later (21–23 days after oocyte retrieval). Scans were repeated weekly until the detection of an embryo heartbeat and monthly thereafter. Micronized intravaginal progesterone (400 mg) (Progeffik®, Laboratories Effik S.A., Madrid, Spain; or Utrogestan®; SEID S.A., Barcelona, Spain) were administered daily from the day of fertilization (Day +1) until the day of the pregnancy test and maintained in pregnant patients until Day 80 of pregnancy.

Ovum retrieval and ICSI

Follicles were gently aspirated and the oocytes were washed in HEPES (LifeGlobal, Canada) and cultured in Global fertilization medium (LifeGlobal, Canada) at 5.0% CO₂ and 37.0°C for 4 h before oocyte denudation. Removal of granulosa cells from the oocyte was carried out by mechanical pipetting in 1:1 hyaluronidase and Global fertilization medium prior to ICSI.

ICSI sperm capacitation was performed in Global fertilization medium and ICSI procedures were performed in a medium containing HEPES. Finally, the zygotes were placed inside a pre-equilibrated culture slide (EmbryoSlide, Unisens FertiTech, Aarhus, Denmark) with 12 droplet wells, each containing 20 μl of Global medium (LifeGlobal, Canada) with 1.4 ml overlay of mineral oil (SAGE, BioCare Europe, Italy) to prevent evaporation. The slides were prepared 24 h in advance and left in the incubator to pre-equilibrate at 5.0% CO₂ and 37.0°C. After pre-equilibration, any bubbles were removed before the zygotes were inserted into individual medium droplets on the slide. Fertilization, as indicated by the appearance
of two pronuclei and two polar bodies, was assessed from time-lapse images obtained by the software from the EmbryoScope™ (Unisense FertiliTech, Aarhus, Denmark) (Tejera et al., 2011).

**Embryo culture**

EmbryoSlides containing zygotes were placed in the EmbryoScope immediately after ICSI and incubated for 5 days with 5.0% CO₂ at 37.0 °C. Images with a resolution of 1000 x 1000 pixels were acquired of each embryo every 20 min at seven different focal planes, starting from the placement of the embryos in the incubator a few hours after ICSI and up to 120 h after fertilization, to determine the exact timing of cell divisions (Cruz et al., 2011).

**Evaluation of time-lapse images**

Retrospective analysis of the acquired images of each embryo was made with an external computer running specialized software, the EmbryoViewer® workstation (Unisense FertiliTech, Aarhus, Denmark), and using image-analysis software in which all the listed embryo developmental events were annotated together with the corresponding timing of events in the hours following ICSI.

Using the EmbryoViewer®, we determined the precise timing of cell divisions and other development parameters including the following: t2, time of cleavage to two-blastomere embryo; t3, time of cleavage to three-blastomere embryo; t4, time of cleavage to four-blastomere embryo; t5, time of cleavage to five-blastomere embryo. The time of cleavage was defined as the time point when the cell division was completed, i.e. the timing of the first time-lapse image where the two new daughter cells were completely segregated and enclosed by their own cytoplasmic membrane. We also determined some variables related to the duration of cell cycles designated cc2 (the second cell cycle), i.e. the duration of period spent as a two-blastomere embryo (t3 – t2); cc3 or the third cell cycle (t5 – t3); s2 or the synchrony of the two divisions from two-blastomere to four-blastomere stage, i.e. the duration of the time spent as 3-cell embryo (t4 – t3).

In order to look for potential embryo time markers, we assessed the percentage of embryos that followed the timing of cleavage-stage divisions, as proposed by Meseguer et al. (2011) as optimal timings: t2: 24.3–27.9 h; t3: 35.4–40.3 h; t5: 48.8–56.6 h; s2: <0.76 h; cc2: <1.19 h, based on the quartiles of the cleavage timing encountered in their study.

We also identified embryos that fulfilled any of the following proposed exclusion criteria: uneven blastomere size at the 2-cell stage, multinucleation at the 4-cell stage and abrupt division from zygote to three-blastomere embryo (cc2 <5 h).

**Morphokinetic categories**

A hierarchical classification procedure has been proposed by Meseguer et al. (2011). Embryos that fulfilled any of the above-mentioned exclusion criteria belong to category E.

The two first levels of this grading procedure were evaluated with t5 as the primary timing variable and s2 as the secondary timing variable; if the value of t5 fell inside the optimal range (48.8–56.6 h), the embryo was graded as A or B; if the value of t5 fell outside the optimal range, the embryo was graded as C or D. If s2 was within the optimal range (<0.76 h), the embryo was graded as A if t5 was inside the optimal range and as C if t5 was outside the optimal range. Similarly, if s2 >0.76 h, the embryo was graded as B if t5 was inside the optimal range, and as D if t5 was outside the optimal range. According to Meseguer et al. (2011) the resulting classification is expected to give the highest implantation for Class A and the lowest for Class D, with intermediate values for Classes B and C. Finally, the embryo was classified with the extra plus (+) if cc2 ≤11.9 h (A+/B+/C+/D+) or with a minus (−) if cc2 >11.9 h (A−/B−/C−/D−).

**Data analysis**

The embryos were divided into three groups based on BMI as described and the values for the timing parameters were compared among the different groups.

Embryos were selected for transfer according to conventional measures of morphological quality on Day 3 (number of cells, embryo fragmentation, blastomere symmetry). The derived morphokinetic parameters and cleavage times were not used in the selection process.

The results were analyzed using the ANOVA test for comparison of means of timings and the $\chi^2$ test or Fisher’s exact test for comparison of proportions. A $P$-value of $<0.05$ was regarded as a statistically significant difference in descriptive parameters, which are represented as one value per patient. However, because we had several embryos from each patient (repeated measures) and thus the $P$-value could be lower than expected from our reported sample size, for embryo analysis we reduced our level of significance to $P = 0.018$ (Meldrum et al., 2011). Statistical analysis was performed using the Statistical Package for the Social Sciences 17.0 (SPSS Inc., Chicago, IL, USA). Type II error (or Beta Risk) was calculated by macro: Macro !NM2IS V2007.12.14 (c) (J.M. Domenech, A. Bonillo and R.Sesma).

**Results**

**Demographics**

Female age was similar for the infertile groups (A and B) but significantly lower in oocyte donors (Group C) and significantly higher in oocyte recipients, as expected (Table I). In Groups A and B tubal pathology, IUFI failure (four or more failed previous attempts) or unknown infertility were the indications for IVF. In oocyte recipients advanced maternal age, low ovarian response in previous IVF attempts, endometriosis or genetic diseases were the indications for treatment. Three of the 13 obese infertile women included (23.1%) presented polycystic ovary syndrome based on the Rotterdam criteria (Rotterdam, 2004). The distribution of ovarian stimulation protocols with GnRH agonists or GnRH antagonists, as well as the use of rFSH, or rFSH plus hMG, for follicular recruitment and development did not differ among the three study groups, and neither did the total dosage of gonadotrophins (data not shown).

The number of oocytes retrieved in the obese group (Group A) was lower than in the other two groups, but with a borderline significance. In addition, fewer mature oocytes were obtained in obese women. However, fertilization rates and number of embryos transferred were similar for the groups, although obese patients also had a significantly smaller number of surplus embryos for cryopreservation (Table I). Implantation, pregnancy and miscarriage rates did not show significant differences. However, sample sizes were not large enough to analyze these outcome parameters properly (Table I).

The average timings of embryo cleavage (from t2 to t5) together with the variables that define the length of the intervals for each group of patients are presented in Table II. The average ‘t’ values show significant differences between embryos derived from normoweight fertile donors (Group C) and those from normoweight infertile or obese infertile women. No differences were observed between normoweight infertile (Group B) and obese infertile women (Group A).
In this case Type II error (or Beta Risk), which is the probability that H1 hypothesis (obese women present differences in embryo morpho-kinetics) occurs, was 25.4% for t5 and 16.1% for s2.

In order to determine whether female obesity affected the proportion of embryos falling into the optimal range, the fraction of embryos inside and outside this range was calculated for Groups A, B and C (Fig. 1). No significant differences were seen between the three groups, although there was a tendency for a reduction in the proportion of optimal embryos when women were infertile and especially obese.

As mentioned under Morphokinetic categories, correlations between morphokinetic parameters and embryo implantation form the basis for a proposed hierarchical classification, as previously described by Meseguer et al. (2011). This model divides all the embryos evaluated into five categories with a decreasing implantation potential from Classes A to E. For the embryo distribution in the algorithm categories, our results were quite similar to those of Meseguer et al. (2011) and related to ‘optimal’ ranges. Although not statistically significant, the embryo allocation in Class E (criteria exclusion) was slightly lower in the normoweight fertile group than in the obese infertile group. The data follow the same pattern in the other categories (from Classes A to D), with more embryos in the optimal range for the normoweight fertile group (Group C). Thus, there is a far higher proportion although not statistically significant of embryos from cycles of the normoweight fertile group in the good prognosis Class A (Table III).

**Discussion**

In this preliminary study we did not detect a deleterious effect of female obesity on the embryo based on the study of embryo morphokinetics.

---

**Table I: Demographic characteristics of the groups in a study of the effects of obesity on morphokinetics in the early embryo following ICSI.**

<table>
<thead>
<tr>
<th></th>
<th>Group A: obese infertile (n = 13)</th>
<th>Group B: normoweight infertile (n = 45)</th>
<th>Group C: normoweight fertile (n = 31)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.7 (SD = 3.4)</td>
<td>33.3 (SD = 2.9)</td>
<td>41.5 (SD = 3.6) recipients</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.9 (SD = 2.5)</td>
<td>21.0 (SD = 1.9)</td>
<td>23.1 (SD = 5.3) donors</td>
<td>0.000</td>
</tr>
<tr>
<td>No oocytes</td>
<td>9.3 (SD = 3.0)</td>
<td>11.9 (SD = 5.4)</td>
<td>13.9 (SD = 4.5)</td>
<td>0.050</td>
</tr>
<tr>
<td>No oocytes MII</td>
<td>6.9 (SD = 2.4)</td>
<td>9.6 (SD = 4.5)</td>
<td>12.5 (SD = 3.8)</td>
<td>0.000</td>
</tr>
<tr>
<td>Fertilization rates (%)</td>
<td>75.2 (SD = 16.8)</td>
<td>74.4 (SD = 21.9)</td>
<td>72.2 (SD = 18.3)</td>
<td>0.857</td>
</tr>
<tr>
<td>No embryos transferred</td>
<td>2.0 (SD = 0.0)</td>
<td>1.7 (SD = 0.5)</td>
<td>1.9 (SD = 0.3)</td>
<td>0.057</td>
</tr>
<tr>
<td>No embryos cryopreserved</td>
<td>2.0 (SD = 1.8)</td>
<td>3.1 (SD = 1.7)</td>
<td>4.2 (SD = 2.2)</td>
<td>0.018</td>
</tr>
<tr>
<td>Implantation rates (%)</td>
<td>37.5 (SD = 37.7)</td>
<td>33.3 (SD = 39.9)</td>
<td>41.7 (SD = 37.3)</td>
<td>0.650</td>
</tr>
<tr>
<td>Pregnancy rates (n/%)</td>
<td>7/53.3 (95% CI: 26.7–80.9)</td>
<td>24/53.3 (95% CI: 37.8–68.3)</td>
<td>20/64.5 (95% CI: 45.4–80.8)</td>
<td>0.446</td>
</tr>
<tr>
<td>Miscarriage rates (n/%)</td>
<td>1/7.7 (95% CI: 0.1–36.0)</td>
<td>4/8.9 (95% CI: 0.6–17.2)</td>
<td>3/9.7 (95% CI: 2.0–25.8)</td>
<td>0.285</td>
</tr>
</tbody>
</table>

MII, metaphase II. Analysis of variance (ANOVA) was used for comparison of means and Fisher’s exact test was used for comparison of proportions.

**Table II: Morphokinetic parameters of the study groups.**

<table>
<thead>
<tr>
<th></th>
<th>Group A: obese infertile (71 embryos)</th>
<th>Group B: normoweight infertile (242 embryos)</th>
<th>Group C: normoweight fertile (111 embryos)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>t2</td>
<td>28.3 (5.4)</td>
<td>28.9 (9.1)</td>
<td>26.5 (3.6)*</td>
<td>0.016</td>
</tr>
<tr>
<td>t3</td>
<td>39.0 (5.5)</td>
<td>38.5 (8.2)</td>
<td>36.2 (6.2)*</td>
<td>0.014</td>
</tr>
<tr>
<td>t4</td>
<td>42.9 (6.9)</td>
<td>41.3 (8.2)</td>
<td>39.0 (6.7)*</td>
<td>0.003</td>
</tr>
<tr>
<td>t5</td>
<td>51.2 (9.7)</td>
<td>51.7 (9.6)</td>
<td>48.3 (8.3)</td>
<td>0.040</td>
</tr>
<tr>
<td>s2</td>
<td>3.3 (5.6)</td>
<td>2.6 (4.6)</td>
<td>2.3 (4.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Cc2</td>
<td>10.8 (5.1)</td>
<td>9.4 (5.5)</td>
<td>9.3 (5.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Cc3</td>
<td>12.0 (5.3)</td>
<td>13.5 (7.9)</td>
<td>12.3 (5.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values presented are cleavage times from a zygote to a 5-cell embryo (t2, t3, t4, t5; cc2, second cell cycle, length of period as a two-blastomere embryo (t3 – t2); cc3, time taken to change from a three-blastomere embryo to a five-blastomere embryo (t5 – t3); t2, synchrony in division from two-blastomere embryos to four-blastomere embryos. Hours: mean (SD). cc2 = t3 – t2; s2 = t4 – t3; cc3 = t5 – t3. ANOVA test was used for comparison of timings between groups A, B and C.
Obesity is a general condition that affects all the tissues and organs in the body causing comorbidities, such as cerebrovascular and cardiovascular diseases, osteoarthritis, type II diabetes, gastroesophageal reflux disease, fatty liver, renal problems, peripheral apnea and cancer (Rao, 2010; Kulie et al., 2011). The reproductive system is also affected with an evident impairment of natural conception and following ART, although the specifics of how obesity alters gametes, embryos and the endometrium are not yet clear.

Some studies have shown a poorer sperm quality and higher infertility rate in obese males (Nguyen et al., 2007; Martini et al., 2010), especially when the female partner is also obese, indicating an impairment of the sperm reproductive potential which is exacerbated when the oocyte or uterine environment is also affected (Ramlau-Hansen et al., 2007). The ovum donation model suggests a clinically reduced endometrial receptivity in obese women (Bellver et al., 2007; Dessolle et al., 2009; DelUgarte et al., 2010), corroborated by gene expression analysis during the window of implantation (Bellver et al., 2011).

Regarding the oocyte and embryo, recent studies carried out in animal models have shown that high glucose concentrations in the cumulus–oocyte complex may affect its metabolic pathways leading to an abnormal maturation of both oocyte nucleus and cytoplasm (Sutton-McDowall et al., 2010). This situation could occur in obese women. Similarly, the increased storage of lipids, such as triglycerides and free fatty acids, in non-adipose tissue cells commonly seen in obese women—the so-called lipotoxicity—may also affect the oocyte by increasing local inflammation, oxidative stress and endoplasmic reticulum stress which would impair ovulation and reduce oocyte competence, defined as the potential of an oocyte for undergoing successful fertilization and subsequent embryo development (Robker et al., 2011).

However, studies performed in obese women undergoing IVF have obtained very different results regarding oocyte and embryo morphological quality. Some reports have shown no negative effect, and some others have identified alterations in several parameters, which vary among studies (reviewed by Bellver, 2008). The largest single-centre study performed to date in the IVF model analyzed the influence of female BMI in IVF, and results included 6500 cycles, 81 581 oocytes and the corresponding embryos. Despite a significant reduction in implantation, pregnancy and live birth rates as BMI increased, no

Table III: Number of embryos in each category of the hierarchical classification tree model (Meseguer et al., 2012) according to BMI and fertility status.

<table>
<thead>
<tr>
<th>Embryo category</th>
<th>Group A: obese infertile (71 embryos)</th>
<th>Group B: normoweight infertile (242 embryos)</th>
<th>Group C: normoweight fertile (111 embryos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.5 (6)</td>
<td>8.3 (20)</td>
<td>14.4 (16)</td>
</tr>
<tr>
<td>B</td>
<td>14.1 (10)</td>
<td>7.9 (19)</td>
<td>8.1 (9)</td>
</tr>
<tr>
<td>C</td>
<td>19.7 (14)</td>
<td>24.4 (59)</td>
<td>27.0 (30)</td>
</tr>
<tr>
<td>D</td>
<td>31.0 (22)</td>
<td>32.2 (78)</td>
<td>27.0 (30)</td>
</tr>
<tr>
<td>E</td>
<td>26.7 (19)</td>
<td>27.2 (66)</td>
<td>23.5 (26)</td>
</tr>
</tbody>
</table>

Data are presented as % (n) for each category. $P = 0.467$. Proportions of embryo categories were compared by the $\chi^2$ test.
significant difference in oocyte or embryo quality parameters was detected (Bellver et al., 2010). Studies suggest that classical static morphological parameters seem not to be useful for analyzing the impact of obesity on embryo quality (Bellver et al., 2010; Shah et al., 2011). Epigenetic modifications of the embryo genome by pregravid or gravid female obesity—the so-called nutritional programming—may also be responsible for some of the described adverse reproductive outcomes in obese women (poor implantation, miscarriage, congenital malformations, intrauterine death), as well as for the increased risk of chronic diseases during childhood, adolescence and adulthood in the offspring, such as obesity, cardiovascular disease, metabolic syndrome or diabetes type II (Jungheim and Moley, 2010). Obviously, these modifications cannot be detected by embryo morphology alone.

Another approach to embryo assessment is the study of embryo dynamics as shown in the present study. Time-lapse technology has been shown to improve embryo selection by adding new useful quality parameters of embryo development timings. In fact, various patterns of embryo cleavage have been related to different success rates of embryo implantation and a hierarchical classification (Meseguer et al., 2011). Our results confirm that there is no significant relationship between female obesity and embryo developmental kinetics. In terms of percentage of ‘optimal’ embryos—the embryos that follow a specific chronological pattern of development which increases its implantation potential—we could find no significant differences for any of the predictive potential variables (t5, c2c and s2) between embryos from obese infertile women and from normoweight infertile women. These results suggest that when using an objective evaluation tool, such as time-lapse imagery, for embryo analysis the likelihood of obtaining a good quality embryo cohort is comparable for obese and non-obese women. There was a trend towards different embryo morphokinetics in normoweight infertile versus obese infertile patients in the analysis of timing values (Fig. 1). However, in order for these differences to reach significance we would need >1000 embryos per category, or even 5000 for some variables, which is difficult to achieve with the current technology and the frequency of obese women in our study population. The low number of obese infertile women in our sample is a limitation of this study.

We saw a significant difference in embryo-cleavage pattern between normoweight fertile women (Group C) and infertile women (both Groups A, obese infertile and B, normoweight infertile) (see Table II). Assuming that early-cleaving embryos are of a higher quality than their later-cleaving counterparts (reviewed by Montag et al., 2011), we observed an impact on embryo development kinetics, with the presence of more optimal embryos in the fertile group. These data are in agreement with the clinical data on morphokinetics which relate specific time ranges to higher implantation probabilities (reviewed by Meseguer et al., 2012) and this is reflected in the clinical outcome parameters presented in Table I.

We are aware that the basic concept of this study was based on embryo morphokinetics only until Day 3 (~72 h), and it could be possible that obesity may play an important role in the late stages of embryo development. However, we have previously compared the blastocyst rate and morphological features for cleavage-stage embryos that were graded according to their morphokinetic development (Cruz et al., 2012): the blastocyst rate and the morphology grading were highest for embryos from the best morphokinetic grade A, and gradually decreased to the lowest rates and qualities for the lowest morphokinetic grade D. Therefore, there appears to be a clear relationship between blastocyst development and morphokinetic grading at the early stages: although purely speculative, we would therefore suggest that if no effects of obesity are observed in the present study, no changes in embryos should be expected at later stages (blastocyst formation and quality). However, the correlation between early cleavage cycles and blastocyst formation was obtained using data from a different patient cohort and although the present data may suggest a similar outcome, the evidence for that is still missing, as ‘obese’ embryos could differ after Day 3.

To our knowledge, this is the first study to explore the developmental characteristics of embryos in a time-lapse system in relation to female obesity. Taking into account that this was a retrospective analysis, a future prospective randomized study will be needed to confirm the hypothesis presented here.

In terms of limitations of the study, the data for clinical outcome parameters should not be considered as definitive as this was not the goal of the study, and the patient sample was not large enough to reach reliable conclusions. In fact, the key objective of the study was to analyze the morphokinetics of embryos and the relationship with BMI in the presence or absence of infertility.

To conclude, this preliminary study using dynamic embryo assessment by time-lapse technology could detect no impairment in embryo quality caused by female obesity.

Acknowledgements

The authors want to thank Kamilla Sophie Pedersen from Unisense Fertitech for her critical reading of the manuscript.

Authors’ roles

J.B. designed the study, selected the cases and wrote the paper. A.M. and N.G. helped in the embryo assessment by time-lapse analysis. L.P. helped in the selection of patients. M.M. performed the embryo assessment and the statistical analysis, and also designed the study and wrote part of the paper.

Funding

No external funding was either sought or obtained for this study.

Conflict of interest

None declared.

References


