Fragmentation of human cleavage-stage embryos is related to the progression through meiotic and mitotic cell cycles

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Objective: To study whether fragmentation of human embryos is related to the progression through meiotic and mitotic cell cycles.

Design: This report consists of two observational studies.

Setting: Not applicable.

Patient(s): A total of 1,943 oocytes from 297 patients and 372 embryos from 100 patients were imaged in the Polscope instrument and monitored in the Embryoscope, respectively.

Intervention(s): Completion of the first meiotic division was determined by visualization of the meiotic metaphase II spindle in human oocytes, and the duration of the first three mitotic cell cycles was determined with time-lapse microscopy. The percentage of embryo fragmentation was recorded 42–45 hours after insemination.

Main Outcome Measure(s): Appearance of the meiotic spindle; durations of the first, second, and third mitoses.

Result(s): Human embryos with a low degree of fragmentation (<10%) at 42–45 hours after insemination originated from oocytes with an early appearance of the meiotic spindle (mean 35.5 hours after hCG injection), early first mitosis (28.2 hours after insemination), late start of the second mitosis (38.0 hours after insemination), and a shorter duration of the third mitosis (1.1 hours). Highly fragmented embryos (>50% fragmentation) originated from oocytes with a late-appearing meiotic spindle (36.5 hours after hCG injection), delayed initiation of the first mitosis (29.8 hours after insemination), early start of the second mitosis (36.4 hours after insemination), and a longer duration of the third mitotic cell cycle (4.1 hours).

Conclusion(s): The observed associations suggest that the process of fragmentation of in vitro-derived embryos was related to the progress of the meiotic and the mitotic cell cycles. (Fertil Steril® 2014;101:1054–1063. ©2014 by American Society for Reproductive Medicine.)

Key Words: Assisted reproduction, fragmentation, meiosis, mitosis, meiotic spindle

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Fragments in early human embryos are anuclear membrane-bound extracellular cytoplasmic structures that may contain sequestered chromosomes (1, 2). The majority of in vitro–produced human embryos display some degree of fragmentation (2, 3). It is well established that increased fragmentation in embryos correlates with reduced implantation and pregnancy rates after in vitro fertilization (IVF) (1, 4–7). Furthermore, moderate to extensive fragmentation is associated with multinucleation of blastomeres and chromosomal abnormalities (2, 8, 9).

However, fragmentation does not seem to correlate with maternal age (10, 11), and fragmentation is not an exclusive determinant of developmental competence, which is also related to the number of blastomeres, cell size, number of nuclei, and symmetry (4, 12–14). Fragmentation has also been observed in embryos of various species in vivo (15–17), indicating that it is neither a species-specific feature nor an artifact of culture in vitro.

The mechanisms leading to embryo fragmentation remain unclear, although several hypotheses have
been put forward. Abnormal cytoplasmic adenosine triphosphate content may affect membrane integrity and promote defects involved in increased fragmentation (18, 19). The role of apoptosis in embryo fragmentation has been controversial (3, 20). Several studies had confirmed the presence of markers of programmed cell death in fragmented embryos, including the cell-death regulatory molecules Bax and Fas and the proapoptotic genes *bokakiri* and *caspase-3* (21–23). Increased TUNEL labeling has been reported after compaction of normally developing embryos (3), and embryos that have intact blastomeres and only minor fragmentation have been found to lack TUNEL labeling (24). Nevertheless, apoptosis is believed to be a consequence rather than a cause of fragmentation (3), and human embryos may undergo fragmentation rather than apoptosis in response to aneuploidy (2).

Notwithstanding the contribution of sperm to embryo development, there is an association among oocyte quality, maturation status, and embryo quality (25, 26). Indeed, the development, there is an association among oocyte quality, maturation status, and embryo quality (25). The oocytes may also be affected by the defects deriving from a delayed maturation, such as higher predisposition to chromosomal abnormalities (28, 29). Reports on induction of fragmentation in oocytes during the cytokinetic phase of the transition from metaphase I (MI) to metaphase II (MII), a prolonged absence of the meiotic spindle is most probably indicative of oocyte abnormality (27, 28). The oocytes may also be affected by the defects deriving from a delayed maturation, such as higher predisposition to chromosomal abnormalities (28, 29). Reports on induction of fragmentation in oocytes during the cytokinetic phase of polar body extrusion imply that fragmentation is due to cytoskeletal disorder in the oocyte and reflects oocyte quality (25).

Time-lapse monitoring systems have become an important tool for clinical embryo assessment. Indeed, it is thought that human embryos with a superior developmental potential might have a characteristic temporal progress through cell divisions (30, 31), and time-lapse systems allow an extended assessment of embryo fragmentation.

The aim of the present study was to explore, with the use of polarized light microscopy and time-lapse recording, whether embryo fragmentation was related to a timely progression through the meiotic and mitotic cell cycles.

**MATERIALS AND METHODS**

**Ovarian Stimulation and Oocyte Retrieval**

Oocytes were collected from women who underwent intracytoplasmic sperm injection (ICSI) treatment for male infertility or other infertility causes with earlier poor fertilization after ordinary in vitro fertilization (IVF). Before follicle aspiration, women received controlled ovarian stimulation according to the luteal-phase GnRH agonist (Suprecur; Sanofi-Aventis) down-regulation protocol and daily injections of either recombinant FSH (Puregon [MSD] or Gonal F [Merck Serono]) or purified urinary gonadotropin (Menopur; Ferring). Final follicular maturation was induced with 6,500 IU recombinant hCG (Ovitrille; Merck Serono). Oocytes were retrieved 34–36 hours later by the vaginal route and under guidance of ultrasound.

Cumulus cells were gently removed 35–37 hours after hCG with the use of denudation pipettes (134–145 μm; Swemed) and HEPES-buffered prewarmed hyaluronidase (Hyase-10 ×; Vitrolife). The oocytes were then evaluated for nuclear maturity and classified as germinal vesicle (GV), MI, MII, or abnormal oocytes. The evaluation was based on the presence of the GV, GV breakdown, or extrusion of the first polar body, respectively.

During ovum pick-up and throughout all subsequent manipulations, the temperature of dishes and tubes was constantly maintained at 37°C with the use of warmed stages or incubators as appropriate. Oocytes were visualized with the use of the LC-Polscope system (Cambridge Research and Instrumentation [CRI]). Time-lapse recording of embryos was performed in the Embryoscope (Unisense Fertilitech).

Oocytes were cultured in a humidified atmosphere of 5% CO₂ and 20% O₂ at 37°C in Universal IVF medium (Origio) or in 6% CO₂ and 20% O₂ at 37°C in G-IVF medium (Vitrolife). Embryos were incubated with 6% CO₂ at 37°C in G1 medium (Vitrolife).

**Visualization of the Meiotic Spindle by Means of the Polscope**

Before ICSI, MII oocytes (n = 1,943) aspirated from 297 women were subjected to polarized light microscopy with the use of the LC-Polscope system. The oocytes were placed in 8-μL droplets of preheated HEPES-buffered holding medium (Origio) covered with prewarmed oil on a glass-bottomed culture dish (Willco Wells). Each dish contained no more than four droplets with one oocyte in each droplet. Oocytes were observed with the Polscope instrument 35.5–40.5 h after hCG. Imaging was performed routinely within 3 hours after ovum pick-up. Observations were performed at 37°C on a heated stage, with the use of a microscope (Carl Zeiss) fitted with the LC-Polscope at ×200 magnification. Integrated image analysis software (Oosight Meta; CRI) was used to visualize the birefringent structures. The oocytes were rotated if necessary with a holding pipette, the appearance of a meiotic spindle was noted, and images were captured. Detailed image analysis was subsequently performed by a single trained observer.

**Intracytoplasmic Sperm Injection Procedure**

ICSI was performed 36–41 hours after hCG in plastic dishes (Falcon; VWR) in 8 μL holding medium (Origio) or G-gamete (Vitrolife) containing polyvinylpyrrolidone (Origio) or ICSI (Vitrolife) for slowing the spermatozoa. The injection was performed with the use of a microscope (Carl Zeiss) with Hoffman modulation contrast and micromanipulators (Eppendorf). Only oocytes that had extruded the first polar body were microinjected. During ICSI, the MII spindle, if present, was avoided when injecting the spermatozoon regardless of polar body orientation. Oocytes without detectable spindles were microinjected with their polar body at the 6 or 12 o’clock position.

After the ICSI procedure, the oocytes were cultured in either 0.5 mL IVF medium (Origio) in 4-well dishes (Nunc)
Assessments of Fertilization, Early Cleavage, and Embryo Quality

Fertilization was assessed 19–20 h post-insemination. Normal fertilization was defined by the appearance of two pronuclei (PN) and two polar bodies. The degree of embryo fragmentation was assessed 42–45 hours post-insemination. Embryo transfer was performed on day 2 after oocyte retrieval. Embryos with > 50% fragmentation or multinucleated embryos were not transferred.

Time-lapse Observations in the Embryoscope

Microinjected MII oocytes (n = 652) from 100 patients were cultured in Embryoslides and underwent time-lapse recording in the Embryoscope. The system consists of an incubator with a built-in microscope and a camera connected to a computer. Images were acquired automatically every 20 minutes in seven focus planes, and all recordings were annotated by a single trained observer. The key cytokinetic events were evaluated and marked as follows: polar body extrusion was set at the first frame when the second polar body appeared in the perivitelline space; fertilization was set at the first frame with apparent pronuclei; two pronuclei and two polar bodies defined a normally fertilized zygote; the first mitotic division was set at the first frame with two cells, and the fragmentation at this point was assessed; and the time points for the second (from two to three blastomeres) and third (from three to four blastomeres) mitotic divisions were recorded. The degree of fragmentation as it evolved during embryo development and before embryo transfer was assessed.

Data Analysis

The exact time of meiotic spindle imaging after hCG injection was determined as the difference between the timestamp of a recorded image file and the time of hCG injection. Observations were binned in 15-minute intervals and the proportion of oocytes with a detectable meiotic spindle calculated for each interval. We reasoned that emergence of the MI spindle is a unidirectional process so that the proportion of oocytes with meiotic spindle at a given time point represents the cumulative frequency of spindle emergence up to that time point. We modeled this cumulative frequency distribution with the sigmoid function $y = \frac{T}{(1 + \exp(a - x)/b)}$, where $a$ denotes ED$_{50}$, the time point with 50% of oocytes having a MII spindle, $T$ the maximal cumulative proportion of oocytes with a spindle, and $b$ the curve slope. Curve fitting was performed with the nls function of R (version 3.0.1; www.r-project.org) with the use of $a = 36.5$, $b = 0.4$, and $T = 0.8$ as starting values. We calculated the maximal absolute difference $D$ between the cumulative frequency distributions and derived critical values for $D_n$ ($n =$ number of patients) with the use of the Kolmogorov–Smirnoff test. Time points for mitotic divisions were compared with the use of the Kruskal–Wallis test among the groups. $P<.05$ was considered to be statistically significant.

As required by Norwegian law, approval was obtained from the Data Protection Officer, Rikshospitalet, Oslo University Hospital, to retrieve anonymized clinical records for this study. As imposed by the Officer, this article is marked “quality assurance” solely to indicate this fact.

RESULTS

We imaged, with the use of polarized light microscopy, 1,943 oocytes that were collected for IVF. Of these oocytes, 1,321 (68%) had extruded the first polar body and had the meiotic spindle visualized at the time of observation. The spindle was positioned at a mean 19° angle from the first polar body (range 0–173°). At ICSI, 1,194 of the 1,943 oocytes (61%) were normally fertilized, displaying two pronuclei and two polar bodies. In the group of oocytes with a detectable spindle at the time of observation, 64% were normally fertilized. Fertilization rate was 57% in the group of oocytes without a detectable spindle ($P<.003$).

Oocytes were imaged once during the period of 35.5–40.5 hours after hCG injection. The proportion of oocytes displaying a metaphase II spindle plateaued at ~38.5 hours after hCG (61%, SE 4.1%), with a slight decrease toward ~40 hours (47%, SE 5.2%; $P=.24$ after controlling for multiple comparisons) and a subsequent return to the plateau levels (65%, SE 7.2%) toward the end of the observation period. The cumulative distribution of spindle emergence was therefore estimated by fitting a sigmoid function on the observed proportion of oocytes displaying a MII spindle (Supplemental Fig. 1, available online at www.fertstert.org).

Next, we wished to relate the timely presence of MII spindle to subsequent embryo fragmentation. Therefore, we categorized embryos according to the percentage of fragmentation determined 42–45 hours after insemination. Embryos with a low degree of fragmentation (0%–10%) were found to originate from oocytes that in >50% of cases had a detectable meiotic spindle 35.5 hours (SE 0.8 h) after hCG injection, implying an early progression of meiosis. Highly fragmented embryos (50%–100%) originated from oocytes that had in 50% of cases a spindle detected 36.2 hours (SE 1.1) after injection ($P<10^{-4}$; Fig. 1; Supplemental Table 1, available online at www.fertstert.org), implying a meiotic delay. Other spindle characteristics, such as retardance and the angle calculated between the first polar body and the meiotic spindle, did not correlate with the degree of fragmentation (Supplemental Table 1).

We also monitored development of 602 microinjected oocytes with time-lapse imaging over 42–45 hours. Of these oocytes, 62% ($n = 372$) were normally fertilized, displaying two pronuclei and two polar bodies. Fragmentation of these embryos was evaluated 42–45 hours after insemination. Increased embryo fragmentation was associated with a specific pattern of developmental asynchrony. Embryos with a high degree of fragmentation (50%–100%) compared with low fragmentation (0%–10%) were characterized by a delayed first division: a significantly earlier start of the second mitosis (8.9 hours vs. 10.8 hours after the first mitosis; $P<.001$); a
significant delay of the third mitosis after the second mitosis (2.2 hours vs. 0.6 hours; \( P < .001 \); Fig. 2; Supplemental Table 2, available online at www.fertstert.org). The summed duration of second and third cell cycles showed no significant correlation with embryo fragmentation (\( P = .69 \); Fig. 2). Neither the fourth or the fifth mitosis correlated with embryo fragmentation (\( P = .66 \); and \( P = .29 \), respectively; Supplemental Fig. 2, available online at www.fertstert.org).

The degree of embryo fragmentation after the first mitosis correlated significantly with the degree of fragmentation after the third mitosis, implying that embryos with a high degree of embryo fragmentation was acquired as early as during the first mitosis (\( r = .75 \); \( P < .001 \); Fig. 3).

**DISCUSSION**

Consistently with maternal determinants of early embryonic cell divisions, we observed that absence of the meiotic MII spindle 36.5 hours after hCG injection was associated with a high degree of embryo fragmentation 42–45 hours after ICSI (Fig. 4). Although the mechanisms that link delayed meiosis to embryo fragmentation remain speculative, it may be plausible to consider whether these mechanisms might also be related to cell cycle defects implicated in oocyte aneuploidy. Indeed, human oocytes have a high prevalence of aneuploidy (32). Oocyte aneuploidy may interfere with chromosome pairing, recombination, and spindle assembly and thus result in a delayed meiotic cell cycle (33). A delay in oocyte maturation in response to hormonal stimulation could be related to an abnormal cytoplasmic maturation having severe consequences on chromosome segregation at meiotic divisions (28). Furthermore, oocytes of aged mice, which are affected by reduced sister chromatid cohesion and increased aneuploidy, may complete meiosis more slowly owing to prometaphase delay compared with oocytes of young mice (34). Nonetheless, aneuploidy of individual oocytes from

**FIGURE 2**

Fragmentation of human embryos cultured in vitro is related to progression through the mitotic cell cycles. Human embryos (\( n = 372 \)) were monitored in a time-lapse system to denote the time to pronuclear formation, to the first mitotic division (cc1), the duration of the second mitotic cell cycle (cc2), and the duration of the third cell cycle (s2). The progression through cell cycles was related to the degree of embryo fragmentation evaluated 42–45 hours after microinjection. The embryos with a high degree of fragmentation (\( > 50 \% \)) had a significantly earlier start of the second mitosis (cc2; 8.9 h vs. 10.8 h; \( P < .001 \)) and significantly longer duration of the third mitosis (s2; 2.2 h vs. 0.6 h; \( P < .001 \)) compared with the embryos with low degree of fragmentation (\( \leq 10 \% \)). If the duration of second and third cell cycle were summed (cc2 + s2), there was no correlation with embryo fragmentation (\( P = .69 \)).

aged mice apparently does not correlate with the progress of meiosis [35], and erroneous chromosome attachments may be insufficient to activate the spindle checkpoint [36]. Furthermore, the environmental toxicant bisphenol A was shown to interfere with spindle formation, delay MII, and induce aneuploidy by nondisjunction of homologous chromosomes [37, 38].

Fragmentation is known to be a characteristic feature of human embryos during its first few cell divisions, when the maternal genome determines development. It appears that around the time of embryonic genome activation, between 4-cell and 8-cell stages, the tendency of human blastomeres to fragment is lost [25]. Early embryos with altered developmental kinetics, including abnormal duration of cell cycles and cytokinesis, are more likely to be fragmented and express cytokinesis-related transcripts at reduced levels, and less often develop into blastocysts. Furthermore, these abnormal embryos often have individual blastomeres that express maternal instead of embryonic transcripts inappropriate for the developmental stage [39]. Mathematical modeling of embryo development also suggests that the capacity to reach the blastocyst stage is already determined at the 1-cell stage [40]. These data may collectively indicate that maternal factors, probably including gene transcripts deposited in the oocytes, determine key features of early embryonic development.

The pattern of embryo fragmentation may also be related to the progression of the mitotic cell cycles during embryo development, because we found fragmentation to be associated with a delayed first mitosis, an earlier start of the second mitosis, and a longer duration of the third cell cycle (s2).

![FIGURE 3](image)

Time-lapse monitoring of human embryos in the Embryoscope. The degree of fragmentation 42–45 hours after microinjection is explored in correlation with key time points during embryo development (n = 372). The degree of fragmentation is also denoted at the first division and thereafter when the fragmentation pattern has changed during embryo development. The last time the fragmentation degree is denoted is when embryo morphology quality is assessed 42–45 hours after insemination. Embryos with high degree of fragmentation are characterized by a delayed first cell division (cc1), an earlier start of the second division (cc2), and a longer duration of the third cell cycle (s2).

mediator of sister chromatid cohesion, DNA double-strand break repair, and transcriptional control [43]. MYBBP1A is a predominantly nucleolar transcriptional regulator involved in rDNA synthesis and p53 activation and which may affect genes controlling chromosomal segregation and cell cycle [44].

Fragmentation is believed to be a dynamic process during early embryo development, as emerging fragments may be re-incorporated into blastomeres [19, 45]. However, we found that embryos with a high degree of fragmentation at the first mitotic cell cycle failed to reduce fragmentation by resorbing fragments; indeed, these embryos appeared highly fragmented when accessing embryo morphology 42–45 hours after microinjection (Fig. 3). This finding is in accordance with reports suggesting that fragmentation at this early stage of embryo development correlates with mitotic errors [2, 39]. It has also been reported that cytoplasmic fragmentation occurs during cell division rather than in interphase cells, implying that fragmentation may be a response to a cytoskeletal or structural dysfunction, such as loss of interplay between the spindle complex and cortical microfilaments [25].

By analyzing the cumulative frequency of spindle emergence, we overcame a notable limitation of analysis of meiotic spindles with polarized light microscopy. Indeed, to avoid detrimental effects of changing pH and temperature on the oocytes, we took images at a single time point during embryo development. Studies that have performed time-lapse imaging of mature human oocytes indicate that after the extrusion of the first polar body, a birefringent strand may be visible closely associated with the polar body. This object, a remnant of the MI spindle, dissolves during telophase I, and it may take a further 40–60 minutes until the MII spindle emerges. Once the MII spindle is formed, however, it remains observable for several hours [27]. With a single image available, we may have overestimated the proportion of mature oocytes displaying a MII spindle. Nonetheless, in only 83 of 1,943 oocytes (4.2%) was the spindle closely associated with, i.e., located within or in the immediate vicinity of, the polar body (data not shown).

Chavez et al. found that parameters of embryo developmental kinetics fall within the reference timing range in only 30% of 4-cell aneuploid embryos, but in 100% of euploid embryos [2]. Even though only eight euploid embryos were observed in that study, it was proposed that the majority of the aneuploid embryos would have kinetic parameters outside the normal range. Others have not found a correlation between embryonic euploidy and progression through early development until the blastocyst stage [46]. It is uncertain whether this contradiction between published data is due to differences in aneuploidy assessment.

Paternal contribution to fragmentation in early embryo development can not be excluded. It has been demonstrated that sperm-derived factors can affect the timing of blastomere division [47], and such an effect on cell division might also affect other processes that control fragmentation [48].
it has been found that histone-modified nucleosomes are enriched in the sperm genome around genes coding for embryo development. This may be instructive for expression of genes, noncoding RNAs, and imprinting of loci that are important in embryo development, and may contribute to totipotency (49). Centrosomes from the sperm play multiple roles in various dysfunctions, such as Parkinson disease, Miller-Dieker syndrome, Bardet-Biedl syndrome, Alstrom syndrome, Cohen syndrome, Meckel-Gruber syndrome, Huntington disease, retinal dystrophy, cystic kidneys, and psychiatric disorders such as schizophrenia (50). Therefore, future research will be important to determine centrosome dysfunctions during embryo development (51).

Time-lapse recording of embryos allow precise timing of important events in early embryo development. One limitation of the present study is that automated time-lapse imaging systems do not allow rotation of the embryos, which may hinder detailed assessment of overlying blastomeres, especially in embryos with a high degree of fragmentation. Furthermore, embryo development is influenced by both exogenous factors that may vary between laboratories, such as temperature, pH, and culture medium, and endogenous patient-related factors (52, 53). Therefore, each laboratory should develop internal reference values for timing parameters. The closed time-lapse imaging systems prevent fluctuation in pH and temperature, but individual embryologists may annotate progression of the embryo development differently. In this study, to exclude interobserver variability, all annotations were performed by a single trained embryologist.

CONCLUSION
With the use of polarized light microscopy of the meiotic spindle and time-lapse monitoring of embryos, we show that fragmentation of in vitro–derived embryos is related to the progress of the meiotic and the mitotic cell cycles, particularly at the time point when the oocyte has a visible MI spindle, the time to the second cell division (three blastomeres), and the duration of the third cell cycle (Fig. 4).

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Proportion of oocytes with a metaphase II spindle visualized with polarized light microscopy, correlated with time after hCG injection and fitting a sigmoid function.

**SUPPLEMENTAL TABLE 1**

Meiotic spindle characterizations and embryo development.

<table>
<thead>
<tr>
<th>Event</th>
<th>0–10%</th>
<th>10%–20%</th>
<th>20%–50%</th>
<th>50%–100%</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>n</td>
<td>512</td>
<td>251</td>
<td>215</td>
<td>217</td>
<td>–</td>
</tr>
<tr>
<td>&lt;36.5 h</td>
<td>11 (57.8)</td>
<td>4 (21.1)</td>
<td>3 (15.8)</td>
<td>1 (5.3)</td>
<td>–</td>
</tr>
<tr>
<td>≥36.5 h</td>
<td>501 (42.6)</td>
<td>247 (21.0)</td>
<td>212 (18.0)</td>
<td>216 (18.4)</td>
<td>–</td>
</tr>
<tr>
<td>Angle from polar body</td>
<td>18.8 ± 23.9</td>
<td>16.8 ± 18.0</td>
<td>17.7 ± 24.0</td>
<td>19.4 ± 20.9</td>
<td>.556</td>
</tr>
<tr>
<td>Retardance</td>
<td>1.29 ± 0.38</td>
<td>1.34 ± 0.38</td>
<td>1.40 ± 0.39</td>
<td>1.36 ± 0.39</td>
<td>.088</td>
</tr>
</tbody>
</table>

Note: Data are presented as mean ± SD or n (%). P values calculated with the use of analysis of variance.

### SUPPLEMENTAL TABLE 2

Timing and duration of embryo developmental events and embryo fragmentation.

<table>
<thead>
<tr>
<th>Event</th>
<th>Embryo fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–10%</td>
</tr>
<tr>
<td>No. of embryos</td>
<td>94</td>
</tr>
<tr>
<td>PB extrusion</td>
<td>3.91 ± 1.67</td>
</tr>
<tr>
<td>cc1a</td>
<td>28.18 ± 4.20</td>
</tr>
<tr>
<td>cc2a</td>
<td>10.81 ± 3.58</td>
</tr>
<tr>
<td>t3a</td>
<td>38.01 ± 4.10</td>
</tr>
<tr>
<td>s2a</td>
<td>1.12 ± 2.01</td>
</tr>
<tr>
<td>t4a</td>
<td>38.86 ± 3.66</td>
</tr>
<tr>
<td>cc3a</td>
<td>5.44 ± 4.94</td>
</tr>
<tr>
<td>t5a</td>
<td>40.65 ± 3.27</td>
</tr>
</tbody>
</table>

Note: Data are presented as mean ± SD hours. PB = polar body.

* Nomenclature according to Meseguer et al. (42).