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1
2 **Title: Evidence that the woman's ovarian cycle is driven by an internal**
3 **circamonthly timing system**

4
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18
19 **Abstract:** The ovarian cycle has a well-established circa-monthly rhythm, but the mechanisms
20 involved in its regularity are unknown. Is the rhythmicity driven by an endogenous clock-like
21 timer, or by other internal or external processes? Here, using two large epidemiological datasets
22 (26,912 cycles from 2,303 European women, and 4,786 cycles from 721 North American
23 women), analyzed with time-series and circular statistics, we find evidence that the rhythmic
24 characteristics of the menstrual cycle are more likely to be explained by an endogenous clock-
25 like driving mechanism than by any other internal or external process. We also show that the
26 menstrual cycle is weakly but significantly influenced by the 29.5-day lunar cycle, and that the
27 phase alignment between the two cycles differs between the European and the North American
28 populations. Given the need to find efficient treatments of subfertility in women, our results
29 should be confirmed in larger populations, and chronobiological approaches to optimize the
30 ovulatory cycle should be evaluated.

31
32
33 **Short title:** The woman's ovarian cycle is driven by an internal circamonthly timing system

34
35 **Teaser:** The menstrual cycle is driven by an internal clock and influenced by the moon cycle.
36

37 **MAIN TEXT**

38 **Introduction**

39 The ovarian cycle, also called the menstrual cycle, has a well-established circa-monthly rhythm
40 in humans (1). The human menstrual cycle, which lasts an average of 29.3 days (2), is indeed an
41 infradian biological rhythm, because it is a recurrent biological process with a longer-than-24-
42 hours period. But is there an endogenous clock-like timer, external to the ovarian cycle itself,
43 that determines its duration? Or, is the length of the menstrual cycle simply the sum of the times
44 required for the pre- and post-ovulatory processes? There is a lack of good evidence to provide a
45 definitive answer to this question. However, methods for numerical analysis of circadian rhythms
46 (3) can be used to look for evidence that some sort of endogenous timing mechanism may be
47 involved.

48 The length of the menstrual cycle of a woman at a given age varies around her personal average
49 duration. Each typical ovulatory cycle consists of three parts or phases, and each phase is
50 dedicated to the realization of a specific process. The first phase, the latency phase (4), begins on
51 the first day of menstruation. The main process that takes place during this period is the selection
52 of a dominant follicle that will later lead to ovulation. It lasts an average of one week, but its
53 duration is more variable than the other phases of the cycle. Typically, the latency phase tends to
54 shorten as the number of reserve follicles in the ovaries decreases as menopause approaches (5).
55 The second phase, known as the fertile window (6), is less variable in duration. The processes of
56 this phase are the growth of the dominant follicle until ovulation, as well as the secretion of fluid
57 mucus in the cervix to receive spermatozoa. The ovulatory follicle grows from 10 to more than
58 20 millimeters in diameter over a relatively fixed period of about a week (7). If the first follicle
59 fails to reach maturity, another follicle takes over, extending the fertile window by several days.
60 The third phase of the cycle, known as the post-ovulatory phase, lasts from the day after
61 ovulation to the day before the next menstrual phase. It is considered to be fairly stable in
62 duration, on average 12-13 days. However, there are variations (8), either physiological, due to
63 the more or less rapid maturation of the corpus luteum (9), or pathological (10). Any change in
64 the total length of the cycle is necessarily due to a change in one or more of these three
65 processes.

66 There is some evidence to suggest that each of the three phases of the menstrual cycles may be
67 under the influence of the circadian timing system (11) (12). Disruption of circadian rhythms is
68 associated with disturbances in menstrual function (13). Some authors have shown an effect of
69 atmospheric pollution on the time of ovulation (14), or on the duration of the post-ovulatory
70 phase (15). Others have described an effect of light exposure (see (16) for review) and shift work
71 (17). However, to date there is no strong or even moderate evidence to support the existence of
72 an endogenous timing system that drives the entire menstrual cycle itself.

73 The observation of certain facts made it possible to emphasize the existence of the internal
74 circadian clock. The first fact was the maintenance of a physiological rhythmicity of almost 24
75 hours, in individuals isolated from external influences (18). This was the first evidence for the
76 existence of an internal circadian clock in humans. The existence of this clock was later
77 confirmed, and its biological function was clarified (19). The period of this internal clock is close
78 to 24 hours (24 h 9 min on average in healthy humans (20)), and with a very narrow spread
79 among individuals (SEM of 1 min). External environmental cues precisely reset and
80 synchronizes the clock to the 24-hour day, with light being the most powerful synchronizer of
81 the human circadian timing system. In the absence of a regular light-dark cycle, the circadian

82 clock drifts on its own period (21),(22), and the biological rhythms no longer exhibit 24h
83 rhythmicity. Under certain conditions, both in animals and in humans, the phenomenon of
84 relative coordination phenomenon can occur, with temporary episodes of synchronization
85 between the circadian clock and its synchronizer (the light-dark cycle), and in-between episodes
86 when the clock jumps from one phase to another or drifts (advances or delays) with a different
87 circadian period (23).

88 In the case of menstrual cycles, if an endogenous clock is involved, then the same phenomena
89 should occur, i.e. the cycle length should be highly stable within individuals and with a narrow
90 spread across individuals (endogenous origin). Synchronizing mechanisms should exist to adapt
91 the cycle (its phase) to a biological need that needs to be synchronized with external conditions,
92 and abrupt changes in menstrual periodicity (a lengthening or shortening of the cycle) should be
93 followed by rapid (jumps) or progressive (relative coordination) adjustment and recovery of the
94 individual endogenous infradian period.

95 A non-endogenous mechanistic alternative for achieving a stable menstrual cycle, within
96 individuals and across the population, could be one that relies on external environmental
97 recurrent events. The theory that the duration of the menstrual cycle and human reproduction is
98 closely related to the length of the lunar cycle has been frequently postulated (24),(25),(26),(27).
99 However, methodological weaknesses or statistical errors have generally called into question the
100 results suggesting a significant relationship (28),(29).

101 It has been suggested that some animals that use the high tides for reproduction have an internal
102 clock of the same period as the lunar cycle (30),(31) and that this internal clock would allow
103 them to anticipate the tidal changes so that their reproductive processes can be synchronized with
104 the tides. Recent results report that a single 'circadian/circatidal' clock is sufficient to entrain
105 behavioral patterns at lunar (tidal) frequencies in bivalves (32). With regards to the menstrual
106 cycle, the existence of a relationship, even a weak one, between the processes of the menstrual
107 cycle and the lunar cycle would indicate the probable existence of an internal timing system and
108 a synchronizing effect of the lunar cycle. Recent work has confirmed the interest in this subject
109 (33).

111 **Specific scope**

112 In this article, we sought to gather evidence, if any, for an endogenous timing mechanism driving
113 the menstrual cycle, and for synchronization with the lunar cycle as a potential external driving
114 mechanism. We used a large dataset of menstrual cycle parameters that were collected in studies
115 conducted in Europe (34). To increase the level of evidence, the analyses were repeated on data
116 collected in other studies conducted in North America (35),(36) (Fig. 1). Statistical methods
117 specifically designed to the study of rhythmic time series were applied to these two datasets. Our
118 analysis focused, on the one hand, on the study of successive cycles (internal timing system),
119 and, on the one other hand, on the relationship between menstruation and lunar phases
120 (synchronizing role).

123 **Results**

124 The age of women in the European cohort at study entry was in the ranges 19 to 24 years, 25 to
125 34, 35 to 39, and 40 to 50 for 545 (24%), 1370 (59%), 263 (11%), and 125 (5%) women,
126 respectively. In the North American cohort, the distribution in these age ranges was slightly
127 different: 246 (35%), 393 (57%), 52 (7%), and 4 (<1%). Most women were <35 years old.

128 Table 1 summarizes the number of cycles and women available for each analysis, with the
129 associated selection criteria, stratified by data region.

130 Fig. 2 shows the distribution of cycle lengths in the two cohorts. For both the European and
131 American populations, the distribution was slightly skewed to the right, with a mode slightly
132 below 30 days. The first quartile, median, and third quartile of cycle lengths in the European
133 cohort were 26, 28, and 30 days, respectively; those in the North American cohort were 27, 29,
134 and 31 days, respectively.

135

136 *Actograms*

137 *Global aspect*

138 Observation of the actograms plotted with each woman's cycle series reveals three characteristic
139 patterns: a) overall long-term stability with occasional jumps; b) overall long-term stability with
140 superimposed oscillations lasting less than a year (resembling relative coordination); c) overall
141 stability with rapid random fluctuations. Fig. 3 shows an example of each of these three types of
142 actograms.

143 The actogram in Fig. 3 (A) shows a stable menstrual rhythmicity from cycle 1 to 7, with a mean
144 cycle length close to the average of 24.6 days. Then, we observe a shift (a.k.a. a “phase jump” in
145 circadian biology) to the right after the cycle 7, which is the result of a cycle that is much longer
146 than the average cycle length. From the 8th to the 20th cycle, the actogram is stable again, with
147 cycle durations close to the average cycle length. The actogram shows a second shift (phase
148 jump) to the right after the 21st cycle, which is again a cycle that is much longer than the average
149 cycle length. Then the cycles show a long-term overall stability until the end (49 cycles), with a
150 period shorter than the average cycle length. This first actogram highlights the need to take into
151 account trends (drifts and jumps) whose duration is long, usually more than one year.

152 The actogram shown in Fig. 3 (B) shows oscillations around a mean cycle length of 27.8 days.
153 These oscillations take the form of a brief drift to the right when the cycles are slightly longer
154 than average (menstrual cycle lengthening), and to the left when the cycles are shorter than
155 average (menstrual cycle shortening). This second actogram shows oscillatory patterns that
156 resemble relative coordination well known in circadian biology, and reveals oscillations of
157 variable duration (period), often less than a year.

158 The actogram shown in Fig. 3 (C) contains only rapid random fluctuations. This random
159 fluctuation is superimposed on a mean cycle length of 29.1 days in this woman. The consecutive
160 cycles are not similar in duration. Short and long cycles alternate in no particular order.

161

162 *Description of oscillations*

163 We used smoothing techniques to quantitatively estimate the visually observed oscillations. In
164 Fig. 4, the brown line shows the result of the smoothing over a period of nearly one year (twelve
165 menstrual cycles). The orange line shows the result of the fine Christiano-Fitzgerald smoothing,

166 which emphasize the short-term oscillations. On the actogram in Fig. 4 (A) we see a wave before
167 the first particularly long cycle (from cycle 1 to cycle 6) and a not very large (low amplitude)
168 wave after this particularly long cycle (from cycle 10 to cycle 14). Another wave occurs after the
169 second particularly long cycle: approximately from cycle 23 to cycle 28. On the actogram in Fig.
170 4 (B), three oscillations appear without being linked to cycles of particular duration. They occur
171 respectively from the seventh to the twelfth cycle, from the fourteenth to the eighteenth, and,
172 more widely, from the twenty-first to the twenty-sixth cycle. The oscillations of the actogram (C)
173 are both numerous and of large amplitude. However, they are quite different from those
174 described in actograms (A) and (B). In fact, the oscillations of the actogram (C) are interspersed
175 with smaller oscillations without any order. The oscillations of in actogram (C) are simply a
176 reflection of a disordered variability in the length of the cycles.

177 Of the cycles of the 425 women in the European cohort, whose series is 18 menstrual cycles or
178 more, 1,846 oscillations of 4 cycles or more were observed, for a mean number of 4.3
179 oscillations per woman. Of these 1,846 oscillations, 890 (48%) were observed in a stable state,
180 906 (49%) after an abrupt cycle lengthening (phase jump), and 50 (3%) were accompanied by
181 fluctuations.

182 The 890 oscillations in a stable state were observed in 338 of 425 women (80%) in the European
183 cohort. Of these, 150 oscillations of the 890 oscillations (17%) had an amplitude of 3 days or
184 more. They were observed in 115 of 425 women (27%).

186 ***Correlation of the durations of successive menstrual cycles***

187 Figure 5 and table 2 show the significance of the correlations between the lengths of successive
188 cycles. The graph on the left shows the significance of the autocorrelation, the graph on the right
189 shows the significance of the partial autocorrelation. The null hypothesis of the absence of
190 second, third and fourth order autocorrelation was rejected ($p < 0.0001$, $p = 0.0004$ and $p = 0.0024$,
191 respectively). The same applies the second, third and fourth order partial autocorrelations
192 ($p = 0.0032$, $p < 0.0001$ and $p = 0.001$, respectively). Only third-order autocorrelation ($p = 0.0084$)
193 and third-order partial autocorrelation ($p = 0.0030$) were statistically significant for the North
194 American dataset (see Fig. 6).

196 **Menstrual day and lunar phase**

197 To investigate the hypothesis that the menstrual cycle is synchronized by, or entrained to, the
198 lunar cycle as an external synchronizer, we tested the relationships between the menstrual and
199 lunar cycles.

200 In the European cohort as a whole, the first day of the menstrual cycle (menstrual day) occurred
201 most frequently in the waxing crescent: 3,533/26,912 (13.1%) (see Table 3). This percentage was
202 only slightly higher than the average expected for an equal distribution among the 8 lunar
203 phases: 100/8 (12.5%). Nevertheless, both Pearson's Chi² test (whose null hypothesis was the
204 uniformity of the distribution of menstruation across the 8 lunar phases) and the Rayleigh test
205 were statistically significant. However, the Rayleigh test applied to the average per woman was
206 not.

207 In the subset of women in the European cohort whose mean cycle length in the series was in the
208 range [28-30], the first day of the menstrual cycle also occurred most frequently at the waxing

209 crescent: 1,440/10,343 (13.9%). The Pearson χ^2 test and the Rayleigh test were statistically
210 significant. The Rayleigh test applied to the average per woman was also statistically significant.

211 In the North American cohort, there was also a small but significant association between the
212 onset of the menstrual cycle and the lunar cycle, but the menstrual cycle most often began at the
213 full moon and not at the waxing crescent as in the European cohort. Only the Rayleigh test was
214 statistically significant for the entire cohort. However, all three tests were statistically significant
215 when applied to women whose average cycle length over the series was within the range [28-30].
216 Table 4 and Fig. 7 show the results of the Monte Carlo test for the European cohort. For the
217 cohort as a whole, the first day of menses showed a significant association with the waxing
218 crescent of the lunar cycle. In the sub-cohort of cycles of women whose average cycle length in
219 the series was within the range [28-30], the higher frequency of menses starting at waxing
220 crescent was confirmed. The same analysis on the North American cohort confirmed the
221 significant association between menses and lunar cycle, the first day of the cycle starting at new
222 moon (Fig. 8).

223

224 **Discussion**

225 In this study, we looked for evidence of an internal timing mechanism driving the menstrual
226 cycle, i.e., that would help determine the duration of the menstrual cycles. To do this, we looked
227 for stability and instability of menstrual cycles, as well as evidence of jumps from one phase to
228 another, and evidence of relative coordination around an intrinsic menstrual period (average
229 cycle duration), as these phenomena are classically described in circadian biology. We also
230 examined the relationships between the menstrual cycle and the lunar cycle. Our aim was to
231 provide additional arguments in favor of 1) an endogenous timing mechanism regulating the
232 length of the menstrual cycle, and 2) an external synchronizing drive, at least in part, by the lunar
233 cycle. Measurements of the partial or separate effects of the two possible causes 1) and 2) are not
234 available, as they would require the use of a complex statistical model and an even larger data set
235 than the one available to us.

236 Our findings include a correlation between the duration of successive menstrual cycles, and a
237 statistically significant relationship between the menstrual cycle and the phases of the lunar
238 rhythm. In what follows, we will discuss to what extent these two observed facts are arguments
239 in favor of the existence of an internal timer (that expresses a near-monthly period) and an
240 external synchronizer (the lunar cycle).

241

242 ***Correlation between the duration of successive cycles***

243 The raster plot graph (arrangement of the cycles on top of each other in the same graph) has been
244 widely used in circadian biology (37). However, we believe that this is the first attempt to
245 visually examine how the menstrual cycle dynamically varies around the mean cycle length (an
246 estimator of the presumed circamonthly infradian period), as is done in the actogram, in 2 large
247 populations of women, and on 2 continents.

248 We found three types of oscillations in the actograms. The first one (Fig. 3A) can be interpreted
249 as a simple construction: after a particularly long cycle, the return to an average cycle length
250 results in a wave on the actogram without particular significance. The phase jumps between one
251 cycle and the next are preceded by a segment of stable oscillations (drifting at a given period),
252 and followed by another segment of stable oscillations (drifting at the same or a different period

253 than before the jump). This is reminiscent of what is observed in chronobiology when the
254 circadian clock is barely synchronized to a particular environmental cycle (at the limit of the
255 circadian clock's range of entrainment), but at some point fails to stay synchronized (because it
256 is outside the range of entrainment), and moves to another stable state (38) (phase jumps). The
257 second type of oscillations we have described (Fig. 3B), observed in a stable state, is important
258 for the topic addressed in this paper. It is the more or less long oscillations that reflect a
259 correlation between the duration of successive cycles. In fact, it is the succession of several
260 cycles slightly longer than the average cycle length that shows a wave to the right on the
261 actogram. And conversely, it is the succession of several cycles slightly shorter than the average
262 that shows a wave to the left. These oscillations suggest that the duration of a cycle depends on
263 the duration of the previous cycles. The third type (Fig. 3C), where the oscillations on the
264 actogram are apparently random fluctuations in cycle length, can also be interpreted as having no
265 particular significance.

266 To verify the significance of this correlation between successive cycle durations, we have
267 estimated the statistical significance of the autocorrelation between successive cycle durations.
268 Indeed, there is an equivalence between a significant, albeit weak, autocorrelation between the
269 duration of successive cycles and the oscillations on the actogram. We observed at least one
270 wave in steady state in the majority of the women in the European dataset who had a series of 18
271 or more consecutive cycles. The statistical significance of the existence of this autocorrelation
272 between consecutive cycles was partially confirmed in the North American dataset. The
273 correlation is sometimes positive and sometimes negative, but on average it is statistically very
274 significantly different from zero.

275

276 ***Partial autocorrelation across multiple cycles, mechanisms and timing***

277 The partial autocorrelation of ranks 2 to 4 was significant. Thus, the autocorrelation between the
278 duration of a cycle and that of the 4 preceding cycles is not simply the reflection of a correlation
279 between two consecutive cycles, but a direct relationship between the duration of a cycle and that
280 of the 4 preceding cycles. We advocate that there are two possible mechanisms for this.

281 The duration of follicular growth, from the primordial follicle to the ovulatory follicle, which is
282 about 90 days, or 3 ovulatory cycles (39), can be translated into a visually stable menstrual cycle
283 on the actograms. Indeed, it is conceivable that the specific hormonal processes involved in the
284 follicular cycle act on the growing passage and that this influences the length of the cycle in
285 which one of them will end up ovulating. With such a homeostatic mechanism, which could be
286 visualized as an hourglass process, we would see a relative stability of the menstrual cycle, and
287 occasional jumps with longer cycle durations when an ovulatory cycle fails before producing a
288 mature follicle that triggers the initiation of a new follicular growth. However, this would not
289 produce shorter cycles, as seen in the relative coordination oscillation we described (Figs. 3A
290 and 3B), but only longer cycles (jumps to longer cycles and/or lengthening of the period of the
291 menstrual cycle).

292 The other possible mechanism, in favor of the existence of a clock-like timer, is evoked by the
293 oscillations of the actogram (Fig. 3B). The slow oscillations around the mean infradian period of
294 the menstrual cycle are reminiscent of what is observed with a spring in action. The more it is
295 stretched or compressed, the more it returns with force to its intrinsic length. This mechanism is
296 also reminiscent of the relative coordination phenomenon described in circadian biology, which
297 reflects both the existence of an endogenous clock and an acute effect of an external

298 synchronizer on the circadian timing system (producing oscillations of phase around the
299 endogenous period). Actually, the fact that the circadian clock can be mathematically modeled
300 by a limit cycle that also explains relative coordination (or the rhythmic scalloping of the
301 circadian phase (40)) is a strong argument for a clock-like drive of the ovulatory cycle. For the
302 menstrual cycle, this suggests the existence of a mechanism that corrects the difference between
303 the current cycle length (current period of the rhythm) and that of the endogenous period of the
304 menstrual cycle: a few shorter cycles would compensate for a series of a few longer cycles, so
305 that the total cycle length would oscillate around the internal period of the menstrual cycle. In the
306 same way that a few days are needed to compensate for a mismatch between the endogenous
307 circadian phase and the day-night alternation after an intercontinental trip, several cycles may be
308 needed to compensate for a discrepancy between the ovulatory cycle and an internal timer after
309 one or more cycles of unusual length have occurred. We believe that the results of this work
310 support such an endogenous clock-like mechanism, but further work is needed to test this
311 hypothesis and unravel the mechanisms.

312

313 ***Relationships with the lunar cycle***

314 We also analyzed the relationship between the menstrual cycle and the phases of the moon. The
315 close resemblance between the length of the lunar cycle and the length of the menstrual cycle has
316 long raised the question of the relationship between these two phenomena. In the scientific
317 literature, we have found publications that show the more frequent occurrence of menstruation
318 after the new moon (25). Others, on the contrary, present data in favor of the occurrence of
319 menstruation during the period when the moon is brighter, or before and after the full moon
320 (24),(26),(41). These contradictory conclusions, added to other articles that have not found any
321 relationships, lead others to conclude that there is no link between the moon and menstruation
322 (29),(42). A recent article, referring to a text from 1806, took up the same assertion and claimed:
323 "No correlation between lunar and menstrual cycles" but the authors simply analyzed the
324 menstrual cycles of a single woman to reach their conclusion (43).

325 In contrast, our analysis of two large and independent dataset suggests the possibility of a
326 moderate but significant temporal association between the lunar cycle and the onset of the
327 menstrual cycle. However, the menstrual cycle began more often at the waxing crescent in
328 Europe, whereas it was at the full moon in North America. We do not have an explanation for
329 this difference between the continents, but we believe that further study of this question is
330 warranted. The differences observed between the European and American data may be due in
331 part to differences in lifestyles between these continents, as well as changes in lifestyles between
332 the time when the European data were collected and the much more recent collection of the
333 American data. It should be noted that European data, collected in different countries and at
334 different times (some European data were collected in the 1960s, others in the 1990s), are
335 heterogeneous in terms of lifestyle. The emerging large databases, containing information on the
336 cycles of several hundred thousand women, thanks to the smartphone applications used by
337 women to record their menstruation, provide additional opportunities to address this question,
338 which we hope will be carefully studied (2),(44),(45).

339 Three mechanisms have been proposed in the literature to explain the effects of the lunar cycle
340 on living species, which occurs in various ways for other physiological processes in other
341 animals.

342 The first is that of an acute effect of light on physiology. Some animals have a different activity
343 according to the phases of the lunar cycle. Full moon nights are a period of vulnerability for
344 some animals, as predators can reach them if they hunt or feed on these nights. In addition, light
345 is known to alter the physiology of all mammals, including humans, even at low light levels
346 (46),(47),(48), and its effect on the human menstrual cycle has been reported (49). The
347 detrimental effects of “light pollution” and the existence of several pieces of work showing the
348 effect of light on the menstrual cycle have led to the proposal that moon light could be
349 responsible for the effect of the lunar cycle on biology (16). However, since the industrial era,
350 very few Europeans and North Americans are likely to be exposed to alternating moonless nights
351 and full moon nights, as nearly all are exposed to artificial light at night (50). Therefore,
352 although we cannot formally exclude it, we believe that the direct effect of moonlight is unlikely
353 to be involved in the synchronization between the lunar cycle and the menstrual cycle that we
354 observe in our study.

355 The second mechanism invoked is that of the gravitational pull of the moon. In a recent article
356 (33), the authors claimed that the gravitational forces of the moon “clearly contribute” to the
357 onset of menses. We cannot completely rule out this mechanistic possibility; however, since the
358 mass of an individual is very small, the gravitational pull of the moon is negligible and has never
359 been unequivocally shown to have any physiological effects.

360 A third mechanism, based on an internal clock oscillating with a period that has evolved to be
361 close to that of the lunar cycle, has been proposed to be involved in several biological organisms
362 (51). These are organisms that live at the edge of the ocean and for which the tides are involved
363 in controlling reproduction (52). As it will be discussed below, such a clock-like system may
364 have been evolutionarily conserved in organisms that no longer live at the edge of the ocean.
365 Whether this circalunar timing system relies on a specific circalunar clock machinery is
366 unknown and cannot be argued on the basis of our descriptive data; however, given that
367 circannual rhythmicities have been shown to depend on diurnal rhythmicity (53), and that tidal
368 rhythms have recently been proposed to rely on a unique clockwork capable of generating both
369 circatidal and circadian rhythms (32), it is conceivable that the circadian timing system is
370 involved in circa-monthly rhythmicities.

371 In some species, a biological phenomenon related to reproduction can occur twice per lunar
372 cycle, at the two phases when the tides are stronger, i.e., when the Sun, Earth and Moon are
373 aligned, at the new moon or at the full moon (54). Thus, the internal infradian timing system can
374 have the same effect at 2 opposite times. A recent elegant study showed that the menstrual cycles
375 of women with a period of more than 27 days were intermittently synchronized with the lunar
376 cycle (33). The article shows series of menstrual cycles that alternate between synchronization
377 with the new moon and synchronization with the full moon, and also describes the relative
378 coordination phenomenon we see in our datasets and discussed earlier. Our work confirms and
379 further extends both the oscillatory nature of the menstrual cycle and the possibility of
380 synchronization at different phases in two larger datasets.

381 What could be the origin of this mechanism? (51) One of them, without having the means to
382 prove it, could be related to a remnant of the evolution of species. It has been shown that
383 biological clocks are of great importance in this embryonic development (55). Our aquatic
384 ancestors gradually came out of the water and may have acquired an internal clock with a period
385 close to that of the lunar cycle at the time when they were confronted with the tides. Then, during
386 the millions of years of hominid evolution, this rhythm may have been active, possibly
387 associated with the lunar night-light cycle. This may have allowed a relative synchronization of

388 the cycles of women living together. It is known that in some species, this synchronization can
389 be favorable for the evolution of the species (56). Was this the case for hominids? This question
390 has been debated (57),(58).

391 What benefits might be derived from knowing that an internal clock drives the length of the
392 menstrual cycle? This question may be related to the emerging interest in personalized
393 therapeutics and circadian medicine (59). If the existence of an internal clock that controls the
394 menstrual cycle is confirmed in further studies, the medical treatment of ovulation disorders
395 could utilize the chronobiological approaches that have proven successful in the treatment of
396 cancer (60), sleep and circadian disorders (61), and depression (62). As one of the potential
397 approaches, chronotherapy and/or bright light therapy could be tested in clinical trials to test
398 their effect on the menstrual cycle and fertility (63),(64).

399

400 In conclusion, we have noted several clues in favor of the existence of an internal timing system
401 acting on the length of the menstrual cycle. The phase jumps, relative co-ordination and partial
402 autocorrelation observed in the menstrual cycle duration of both European and American cohorts
403 support the existence of a cycle duration regulation with “memory” of the duration of previous
404 cycles, which we interpret as best explained by a circa-monthly timing system of endogenous
405 origin. The identified links between the lunar and menstrual cycles, although weak and
406 inconsistent in terms of phase relationship between the European and North American cohorts,
407 may lead to the periodicity of menstruation, and thus ovulation, as a phenomenon possibly
408 directly influenced by the lunar cycle, or as a remnant of the evolutionary past. It can be treated
409 as additional evidence for an internal near-monthly timing system with the lunar cycle as an
410 external weak synchronizer.

411 Further research into the genetic regulation of the menstrual cycle will be important in
412 understanding the chronobiology of the menstrual cycle. A variant of a gene involved in the
413 regulation of follicular stimulating hormone was found to be associated with a change in cycle
414 length (65). This shows that cycle length is genetically determined at some molecular level, but
415 does not explain how the length of cycles could depend on the length of previous cycles, nor
416 does it show how an internal timing system could affect the length of the menstrual cycle.
417 Clinical factors are also known to correlate with cycle length, including age, parity, and age at
418 menarche (66).

419 Given the need to find effective treatments for subfertility in women, our results should be
420 confirmed in larger populations and the mechanisms deciphered from both human and animal
421 studies. It would then be legitimate to study chronobiological approaches to optimize the
422 ovulatory cycle, either by optimizing its stability or by improving its success. Such time-
423 dependent therapeutic approaches have proven successful in many fields and could also prove
424 effective here.

425

426 **Materials and Methods**

427 **Datasets**

428

429 ***European dataset***

430 The European dataset used in this study was compiled by the Department of Statistics of Padova
431 (Italy) under the direction of Bernardo Colombo (67–71).

432 This database contains information on 51,013 cycles from 3,296 women from Great Britain,
433 Italy, France, Germany and Belgium, and includes five sets of data, as shown in Fig. 1. The
434 database has undergone consistency checks and has been used for several studies on the
435 menstrual cycle (72, 73), fecundity (71, 74–76), and the evolution of the menstrual cycle with
436 age (77, 78). The data were obtained from women with no known cycle disorders.

437 In the database, the cycles were classified in order of occurrence and grouped into series of
438 consecutive cycles. The same woman could participate in several series, interrupted by
439 pregnancy or temporary interruption of data collection for other reasons. The outcome of the
440 cycle, including the possible occurrence of pregnancy, was recorded. All these cycles were
441 natural, without treatment with hormonal contraceptives or ovarian stimulation.

442 For this study we have kept only the series of cycles for each woman between 20 and 50 years of
443 age, whose first cycle was recorded between 1960 and 1997 inclusive. The series were stopped
444 with the cycle preceding the pregnancy cycle or with other events that resulted in a cycle longer
445 than 60 days. Only the first series of consecutive cycles for each woman was finally included.

446 The European dataset, merged into a single file, includes 26,912 cycles from 2,303 women for
447 this study. Details of the number of women and cycles per dataset are given in Table 1.

448

449 *North American dataset*

450 The North American datasets were used for studies of female participants with no known health
451 conditions that would cause cycle abnormalities.

452 The first cohort was recruited from April 2008 to December 2010. Inclusion criteria for female
453 partners of couple participants were that they had to be between 18 and 42 years of age, have a
454 reported menstrual cycle of 21–42 days, have no history of hormonal contraceptive use in the
455 past 3 months and, if post-breastfeeding, have experienced at least three cycles after weaning.
456 More details about this cohort can be found in the original publication (35). Only one series of
457 consecutive cycles were included. The series was stopped with the cycle preceding a pregnancy
458 or any other event that resulted in a cycle longer than 60 days. This dataset, designated
459 Marquette on in Fig. 1, contains one series of consecutive menstrual cycles each for 159 women
460 (total: 1,649 cycles).

461 The second cohort includes the menstrual cycles of women in the United States and Canada with
462 no known health conditions that would cause cycle abnormalities who were enrolled in three
463 different cohort studies (1990–2013). Eligibility criteria in the original studies included women
464 18–40 years of age, not pregnant at entry, having regular menstrual bleeding, and not
465 breastfeeding or, if breastfeeding, not exclusively doing so. More details about this cohort can be
466 found in the original publication (36). The series was stopped with the cycle preceding a
467 pregnancy or any other event resulting in a cycle longer than 60 days. This dataset, designated
468 Utah in Fig. 1, contains one series of consecutive menstrual cycles each for 562 women (total:
469 3,137 cycles).

470 Thus, the North American dataset includes 4,786 (1,649+3,137) cycles from 721 (159+562)
471 women.

472

473 **Ethics:** All datasets were obtained from studies that adhered to the tenets of the Declaration of
474 Helsinki, and participants provided their informed consent to the use of their data. The European
475 dataset is publicly available (34) and all necessary approvals were obtained from the University
476 of Padova. The Utah dataset was collected in a study approved by the University of Utah IRB
477 and local site IRBs. The dataset designated Marquette was collected in a study registered in the
478 Clinical Trial Database under #NCT00843336, and which received IRB approval through the
479 University Office of Research Compliance of Marquette University.

480

481 **Factors studied and their availability in the datasets**

482 Our unit of analysis was the menstrual cycle, with constant fluctuation of duration over time. We
483 considered the woman's self-reported first day of each menstrual bleeding as the first day of the
484 cycle and the cycle time marker. Each cycle lasted up to and including the day before the first
485 day of the next menstrual cycle, which was used to calculate the cycle length.

486 Our outcomes of interest included periodicity and oscillations in the menstrual cycle length,
487 autocorrelations of successive menstrual cycle lengths, and correlations of the first day of menses
488 with the lunar cycle.

489

490 **Statistical analysis**

491 We applied procedures classically used to analyze circadian rhythms. This analysis included the
492 creation of actograms, time series analysis methods, and circular statistics.

493

494 ***Actograms***

495 We created an actogram (also called a raster plot) for each woman. It consists of a series of
496 superimposed lines, each line representing the length of a single menstrual cycle, and each new
497 cycle is represented by a line below that of the previous cycle (Fig. 1). Note that we have named
498 these raster plots "actograms" by analogy between the cycles of physical activity and the cycles
499 of ovulatory/menstrual activity.

500 We chose to create actograms normalized to the average cycle length of each woman, as an
501 estimate of the infradian period (modulo the menstrual cycle's period). This allows each
502 menstrual cycle be visually compared to the average length of a woman's cycle. This average is
503 considered her reference menstrual cycle's period (analogous to the 24-hour duration on
504 actograms used in the study of circadian rhythms). If one menstrual cycle is shorter, the next one
505 starts before the reference period. If one cycle is longer, it is the other way around, the next one
506 starts later in the reference period. As is usually the case with actograms, the advance or delay
507 with respect to the reference period is made visible by a shift of the same duration.

508 Mathematically, this is obtained by calculating the cumulative sum (cumsum) of the differences
509 between the duration of each cycle and the duration of the reference period, i.e. the average
510 duration of menstrual cycles.

511 The actogram is a graphical representation of a time series. In time series analysis, it is common
512 to distinguish between drifts, called trends, and oscillations, also called wavelets. Each wavelet
513 has a duration and an amplitude. To measure these durations and amplitudes in the menstrual
514 cycles, we used a smoothing over a period of nearly one year (twelve cycles), by a kernel

515 smoothing method (79), then we used a Christiano-Fitzgerald type filter to estimate the wavelets
516 (80). Calculation of the difference between these two smoothings, one global over a period of the
517 order of a year, the other local to follow the wavelets consecutively, gives the length and the
518 amplitude of each wavelet.

519 Wavelet durations and amplitudes were calculated for all women with a series of 18 cycles or
520 more. Shorter series are not sufficient for correct estimation. Only oscillations with 4 or more
521 cycles were considered for further analysis, and shorter ones were considered irregularities rather
522 than oscillations.

523

524 ***Time series analysis***

525 To quantify the similarity between the duration of consecutive cycles of the same woman, we
526 first calculated the correlation between each cycle length and the six previous cycles. To do this,
527 we used an autocorrelation function (ACF) (81). The ACF is a function that gives us values of
528 the autocorrelation of any series with its lagged values. Part of the correlation between two
529 cycles separated by a few months may be direct, and the other part may be from one cycle to the
530 next. The ACF does not distinguish between these two types of relationship. Thus, to quantify
531 the direct correlation between two cycles separated by one or more cycles, we used a partial
532 autocorrelation function (PACF). This means that a partial autocorrelation of, for example, ranks
533 2 to 4 would be in favor of a direct relationship between the duration of a cycle and the duration
534 of the preceding 2 to 4 cycles. Here, direct means that it is not the effect of a change in the length
535 of the intermediate cycles.

536 The order in a temporal autocorrelation is defined as the distance between the two times at which
537 the correlation is calculated. Thus, a second-order autocorrelation is the correlation between two
538 cycles separated by one cycle, while a first-order autocorrelation is the correlation between two
539 consecutive cycles. A second or third order autocorrelation may be statistically significant
540 without the lower order autocorrelation being significant.

541 We applied these ACF and PACF functions to the series of consecutive cycles of all women in
542 the European dataset whose series were at least 13 cycles long (i.e., one year or more). We
543 retained all series of at least 7 cycles for the North American database because we had shorter
544 series for this database. However, these shorter series give less precise estimates.

545 We used a Monte Carlo permutation method to test for the existence of a relationship between
546 successive cycles: the observed autocorrelation was compared with that obtained by swapping
547 the cycles of each woman (82). This makes it possible to relate the observed correlation to the
548 correlation calculated on the same cycles, but arranged in other order. This was repeated 5000
549 times. Then, the average autocorrelation observed on all the women was compared to these 5000
550 average autocorrelations calculated on swapped data. The position of the observed value among
551 the 5000 simulated values gave the p-value, which can be interpreted as the probability that an
552 observed autocorrelation between the cycles for each woman occurred by random chance alone.

553 We then plotted a graph showing the average correlations on swapped data and the observed
554 average correlations. For comparability of results, we used the z-score as a unit: a deviation from
555 the mean of more than 2 standard deviations reflects a threshold for statistical significance.

556

557 ***Circular statistics***

558 The eight lunar phases we considered were new moon, waxing crescent, first quarter, waxing
559 gibbous, full, waning gibbous, last quarter and waning crescent. These eight phases can be
560 considered as categories, and therefore a Pearson's χ^2 was used to test the heterogeneity of the
561 occurrence of the beginning of the menstrual cycle compared to the lunar cycle.

562 Furthermore, a circular statistics test that takes into account the order of appearance of these
563 eight lunar phases will generally be more powerful to reject the null hypothesis of no association
564 between lunar phase and the date of the first day of the menstrual cycle. We used the circular
565 statistics test called Rayleigh test. It was first applied on all cycles, without taking into account
566 the statistical non-independence between cycles of the same woman. In a second step, we took
567 into account the clustering of cycles for each woman. To do this, we first made a circular average
568 of the lunar phases of occurrence of first day of menstruation of each woman, and then applied
569 the Rayleigh test to these averages. Then, we used the Monte Carlo method again, following the
570 method proposed for the data grouped by the circular method (in our case, the cycles grouped by
571 woman) (83). For the cycles of each woman, the simulated data were generated as follows: to the
572 date of the first day of the first cycle of the series, a number randomly selected between zero and
573 twenty-nine (days) was added. This places the beginning of the first cycle at any point in the
574 lunar cycle. The observed duration of the cycles was then kept unchanged. This was done for
575 each woman in the sample. This process was repeated 5000 times, resulting in many simulated
576 samples. For each of them, the total number of cycles starting at each of the 8 phases of the lunar
577 cycle was calculated. Finally, the observed frequency was compared to the 5000 simulated
578 frequencies to calculate the p-value for each of the 8 phases of the lunar cycle. This p value can
579 be interpreted as the probability of no association between the first day of menstruation and the
580 lunar cycle.

581 In a sensitivity analysis for the circular statistics, the data were restricted to cycles of 28-30 days
582 to more likely correlate with the lunar cycle.

583 The observed data are presented in the form of a pie chart. To facilitate reading, an image of each
584 lunar phase has been placed on the graph with some modification of the source data (84).

585 All calculations were performed using the R software (R version 4.0.3 The R Foundation for
586 Statistical Computing). The significance level we used was 0.05. For bilateral tests with two
587 alternative hypotheses, the threshold was 0.025. Unless otherwise stated, the Bonferroni
588 correction was applied to all tests.

589

590 **References**

- 591 1. L. Rensing, U. Meyer-Grahe, P. Ruoff, Biological timing and the clock metaphor:
592 oscillatory and hourglass mechanisms. *Chronobiol. Int.* **18**, 329–369 (2001).
- 593 2. J. R. Bull, S. P. Rowland, E. B. Scherwitzl, R. Scherwitzl, K. G. Danielsson, J. Harper, Real-
594 world menstrual cycle characteristics of more than 600,000 menstrual cycles. *NPJ Digit.*
595 *Med.* **2**, 83 (2019).
- 596 3. R. Refinetti, G. C. Lissen, F. Halberg, Procedures for numerical analysis of circadian
597 rhythms. *Biol. Rhythm Res.* **38**, 275–325 (2007).
- 598 4. R. Ecochard, Heterogeneity in fecundability studies: issues and modelling. *Stat. Methods*
599 *Med. Res.* **15**, 141–160 (2006).
- 600 5. E. A. Lenton, B. M. Landgren, L. Sexton, R. Harper, Normal variation in the length of the
601 follicular phase of the menstrual cycle: effect of chronological age. *Br. J. Obstet. Gynaecol.*

- 602 **91**, 681–684 (1984).
- 603 6. A. J. Wilcox, D. Dunson, D. D. Baird, The timing of the “fertile window” in the menstrual
604 cycle: day specific estimates from a prospective study. *BMJ.* **321**, 1259–1262 (2000).
- 605 7. R. T. Mikolajczyk, J. B. Stanford, R. Ecochard, Multilevel model to assess sources of
606 variation in follicular growth close to the time of ovulation in women with normal fertility: a
607 multicenter observational study. *Reprod. Biol. Endocrinol. RBE.* **6**, 61 (2008).
- 608 8. K. C. Schliep, S. L. Mumford, A. O. Hammoud, J. B. Stanford, K. A. Kissell, L. A. Sjaarda,
609 N. J. Perkins, K. A. Ahrens, J. Wactawski-Wende, P. Mendola, E. F. Schisterman, Luteal
610 phase deficiency in regularly menstruating women: prevalence and overlap in identification
611 based on clinical and biochemical diagnostic criteria. *J. Clin. Endocrinol. Metab.* **99**, E1007-
612 1014 (2014).
- 613 9. R. Ecochard, T. Bouchard, R. Leiva, S. Abdulla, O. Dupuis, O. Duterque, M. Garmier
614 Billard, H. Boehringer, C. Genolini, Characterization of hormonal profiles during the luteal
615 phase in regularly menstruating women. *Fertil. Steril.* **108**, 175-182.e1 (2017).
- 616 10. J. W. Goldzieher, R. L. Young, Luteal phase deficiency. *Fertil. Steril.* **51**, 197–198 (1989).
- 617 11. L. Yan, R. Silver, Neuroendocrine underpinnings of sex differences in circadian timing
618 systems. *J. Steroid Biochem. Mol. Biol.* **160**, 118–126 (2016).
- 619 12. M. T. Sellix, Circadian clock function in the mammalian ovary. *J. Biol. Rhythms.* **30**, 7–19
620 (2015).
- 621 13. F. C. Baker, H. S. Driver, Circadian rhythms, sleep, and the menstrual cycle. *Sleep Med.* **8**,
622 613–622 (2007).
- 623 14. L. Giorgis-Allemand, J. C. Thalabard, L. Rosetta, V. Siroux, J. Bouyer, R. Slama, Can
624 atmospheric pollutants influence menstrual cycle function? *Environ. Pollut. Barking Essex*
625 *1987.* **257**, 113605 (2020).
- 626 15. A. Merklinger-Gruchala, G. Jasienska, M. Kapiszewska, Effect of Air Pollution on
627 Menstrual Cycle Length-A Prognostic Factor of Women’s Reproductive Health. *Int. J.*
628 *Environ. Res. Public Health.* **14**, 816 (2017).
- 629 16. M. L. Barron, Light exposure, melatonin secretion, and menstrual cycle parameters: an
630 integrative review. *Biol. Res. Nurs.* **9**, 49–69 (2007).
- 631 17. M. Attarchi, H. Darkhi, M. Khodarahmian, M. Dolati, M. Kashanian, M. Ghaffari, E.
632 Mirzamohammadi, S. Mohammadi, Characteristics of menstrual cycle in shift workers.
633 *Glob. J. Health Sci.* **5**, 163–172 (2013).
- 634 18. N. Kleitman, *Sleep and Wakefulness* (University of Chicago Press, Chicago, IL, 1987;
635 <https://press.uchicago.edu/ucp/books/book/chicago/S/bo27408234.html>).
- 636 19. T. A. Bargiello, M. W. Young, Molecular genetics of a biological clock in *Drosophila*. *Proc.*
637 *Natl. Acad. Sci. U. S. A.* **81**, 2142–2146 (1984).
- 638 20. J. F. Duffy, S. W. Cain, A.-M. Chang, A. J. K. Phillips, M. Y. Münch, C. Gronfier, J. K.
639 Wyatt, D.-J. Dijk, K. P. Wright, C. A. Czeisler, Sex difference in the near-24-hour intrinsic
640 period of the human circadian timing system. *Proc. Natl. Acad. Sci. U. S. A.* **108 Suppl 3**,
641 15602–15608 (2011).
- 642 21. J. Aschoff, Circadian parameters as individual characteristics. *J. Biol. Rhythms.* **13**, 123–131
643 (1998).
- 644 22. C. Gronfier, K. P. Wright, R. E. Kronauer, C. A. Czeisler, Entrainment of the human
645 circadian pacemaker to longer-than-24-h days. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 9081–
646 9086 (2007).
- 647 23. R. A. Wever, Light effects on human circadian rhythms: a review of recent Andechs
648 experiments. *J. Biol. Rhythms.* **4**, 161–185 (1989).

- 649 24. W. B. Cutler, Lunar and menstrual phase locking. *Am. J. Obstet. Gynecol.* **137**, 834–839
650 (1980).
- 651 25. S. P. Law, The regulation of menstrual cycle and its relationship to the moon. *Acta Obstet.*
652 *Gynecol. Scand.* **65**, 45–48 (1986).
- 653 26. E. Friedmann, Menstrual and lunar cycles. *Am. J. Obstet. Gynecol.* **140**, 350 (1981).
- 654 27. Y. Komada, M. Sato, Y. Ikeda, A. Kami, C. Masuda, S. Shibata, The Relationship between
655 the Lunar Phase, Menstrual Cycle Onset and Subjective Sleep Quality among Women of
656 Reproductive Age. *Int. J. Environ. Res. Public Health.* **18**, 3245 (2021).
- 657 28. R. G. Foster, T. Roenneberg, Human responses to the geophysical daily, annual and lunar
658 cycles. *Curr. Biol. CB.* **18**, R784–R794 (2008).
- 659 29. I. Ilias, F. Spanoudi, E. Koukkou, D. A. Adamopoulos, S. C. Nikopoulou, Do lunar phases
660 influence menstruation? A year-long retrospective study. *Endocr. Regul.* **47**, 121–122
661 (2013).
- 662 30. A. Takemura, M. S. Rahman, Y. J. Park, External and internal controls of lunar-related
663 reproductive rhythms in fishes. *J. Fish Biol.* **76**, 7–26 (2010).
- 664 31. Y. Takeuchi, R. Kabutomori, C. Yamauchi, H. Miyagi, A. Takemura, K. Okano, T. Okano,
665 Moonlight controls lunar-phase-dependency and regular oscillation of clock gene
666 expressions in a lunar-synchronized spawner fish, Goldlined spinefoot. *Sci. Rep.* **8**, 6208
667 (2018).
- 668 32. D. Tran, M. Perrigault, P. Ciret, L. Payton, Bivalve mollusc circadian clock genes can run at
669 tidal frequency. *Proc. Biol. Sci.* **287**, 20192440 (2020).
- 670 33. C. Helfrich-Förster, S. Monecke, I. Spiouzas, T. Hovestadt, O. Mitesser, T. A. Wehr,
671 Women temporarily synchronize their menstrual cycles with the luminance and gravimetric
672 cycles of the Moon. *Sci. Adv.* **7**, eabe1358 (2021).
- 673 34. Dataset, (available at <https://www.stat.unipd.it/ricerca/basi-di-dati>).
- 674 35. R. J. Fehring, M. Schneider, K. Raviele, D. Rodriguez, J. Pruszynski, Randomized
675 comparison of two Internet-supported fertility-awareness-based methods of family planning.
676 *Contraception.* **88**, 24–30 (2013).
- 677 36. S. Najmabadi, K. C. Schliep, S. E. Simonsen, C. A. Porucznik, M. J. Egger, J. B. Stanford,
678 Menstrual bleeding, cycle length, and follicular and luteal phase lengths in women without
679 known subfertility: A pooled analysis of three cohorts. *Paediatr. Perinat. Epidemiol.* **34**,
680 318–327 (2020).
- 681 37. R. F. Vollman, *The menstrual cycle* (W. B. Saunders Co, Philadelphia, 1977), *Major*
682 *problems in obstetrics and gynecology*.
- 683 38. R. A. Wever, Characteristics of circadian rhythms in human functions. *J. Neural Transm.*
684 *Suppl.* **21**, 323–373 (1986).
- 685 39. A. Gougeon, Human ovarian follicular development: from activation of resting follicles to
686 preovulatory maturation. *Ann. Endocrinol.* **71**, 132–143 (2010).
- 687 40. C. H. Johnson, J. A. Elliott, R. Foster, Entrainment of circadian programs. *Chronobiol. Int.*
688 **20**, 741–774 (2003).
- 689 41. W. B. Cutler, W. M. Schleidt, E. Friedmann, G. Preti, R. Stine, Lunar influences on the
690 reproductive cycle in women. *Hum. Biol.* **59**, 959–972 (1987).
- 691 42. J. Pochobradsky, Independence of human menstruation on lunar phases and days of the
692 week. *Am. J. Obstet. Gynecol.* **118**, 1136–1138 (1974).
- 693 43. B. Lemmer, No correlation between lunar and menstrual cycle - an early report by the french
694 physician J. A. Murat in 1806. *Chronobiol. Int.* **36**, 587–590 (2019).
- 695 44. L. Faust, D. Bradley, E. Landau, K. Noddin, L. V. Farland, A. Baron, A. Wolfberg, Findings

- 696 from a mobile application-based cohort are consistent with established knowledge of the
697 menstrual cycle, fertile window, and conception. *Fertil. Steril.* **112**, 450-457.e3 (2019).
- 698 45. L. Symul, K. Wac, P. Hillard, M. Salathé, Assessment of menstrual health status and
699 evolution through mobile apps for fertility awareness. *NPJ Digit. Med.* **2**, 64 (2019).
- 700 46. G. V. Vartanian, B. Y. Li, A. P. Chervenak, O. J. Walch, W. Pack, P. Ala-Laurila, K. Y.
701 Wong, Melatonin Suppression by Light in Humans Is More Sensitive Than Previously
702 Reported. *J. Biol. Rhythms.* **30**, 351–354 (2015).
- 703 47. A. S. Prayag, R. P. Najjar, C. Gronfier, Melatonin suppression is exquisitely sensitive to
704 light and primarily driven by melanopsin in humans. *J. Pineal Res.* **66**, e12562 (2019).
- 705 48. A. Takemura, S. Ueda, N. Hiyakawa, Y. Nikaido, A direct influence of moonlight intensity
706 on changes in melatonin production by cultured pineal glands of the golden rabbitfish,
707 *Siganus guttatus*. *J. Pineal Res.* **40**, 236–241 (2006).
- 708 49. A. A. Putilov, K. V. Danilenko, A. Y. Protopopova, D. F. Kripke, Menstrual Phase
709 Response to Nocturnal Light. *Biol. Rhythm Res.* **33**, 23–38 (2002).
- 710 50. F. Falchi, P. Cinzano, D. Duriscoe, C. C. M. Kyba, C. D. Elvidge, K. Baugh, B. A. Portnov,
711 N. A. Rybnikova, R. Furgoni, The new world atlas of artificial night sky brightness. *Sci.*
712 *Adv.* **2**, e1600377 (2016).
- 713 51. G. Andreatta, K. Tessmar-Raible, The Still Dark Side of the Moon: Molecular Mechanisms
714 of Lunar-Controlled Rhythms and Clocks. *J. Mol. Biol.* **432**, 3525–3546 (2020).
- 715 52. H. Caspers, Spawning periodicity and habitat of the palolo worm *Eunice viridis* (Polychaeta:
716 Eunicidae) in the Samoan Islands. *Mar. Biol.* **79**, 229–236 (1984).
- 717 53. G. A. Lincoln, I. J. Clarke, R. A. Hut, D. G. Hazlerigg, Characterizing a mammalian
718 circannual pacemaker. *Science.* **314**, 1941–1944 (2006).
- 719 54. H. Ma, M. S. Yang, Y. T. Zhang, H. T. Qiu, X. X. You, S. X. Chen, W. S. Hong,
720 Expressions of melanopsins in telencephalon imply their function in synchronizing
721 semilunar spawning rhythm in the mudskipper *Boleophthalmus pectinirostris*. *Gen. Comp.*
722 *Endocrinol.* **315**, 113926 (2022).
- 723 55. M. Ebisuya, J. Briscoe, What does time mean in development? *Dev. Camb. Engl.* **145**,
724 dev164368 (2018).
- 725 56. M. K. McClintock, Menstrual Synchrony and Suppression. *Nature.* **229**, 244–245 (1971).
- 726 57. C. Power, V. Sommer, I. Watts, The Seasonality Thermostat: Female Reproductive
727 Synchrony and Male Behavior in Monkeys, Neanderthals, and Modern Humans.
728 *PaleoAnthropology.* **2013**, 33–60 (2013).
- 729 58. B. I. Strassmann, The Biology of Menstruation in *Homo Sapiens*: Total Lifetime Menses,
730 Fecundity, and Nonsynchrony in a Natural-Fertility Population. *Curr. Anthropol.* **38**, 123–
731 129 (1997).
- 732 59. J. Taillard, C. Gronfier, S. Bioulac, P. Philip, P. Sagaspe, Sleep in Normal Aging,
733 Homeostatic and Circadian Regulation and Vulnerability to Sleep Deprivation. *Brain Sci.*
734 **11**, 1003 (2021).
- 735 60. E. Ortiz-Tudela, A. Mteyrek, A. Ballesta, P. F. Innominato, F. Lévi, Cancer
736 chronotherapeutics: experimental, theoretical, and clinical aspects. *Handb. Exp. Pharmacol.*,
737 261–288 (2013).
- 738 61. P. C. Zee, S. M. Abbott, Circadian Rhythm Sleep-Wake Disorders. *Contin. Minneap. Minn.*
739 **26**, 988–1002 (2020).
- 740 62. P. A. Geoffroy, C. M. Schroder, E. Reynaud, P. Bourgin, Efficacy of light therapy versus
741 antidepressant drugs, and of the combination versus monotherapy, in major depressive
742 episodes: A systematic review and meta-analysis. *Sleep Med. Rev.* **48**, 101213 (2019).

- 743 63. K. V. Danilenko, O. Y. Sergeeva, Immediate effect of blue-enhanced light on reproductive
744 hormones in women. *Neuro Endocrinol. Lett.* **36**, 84–90 (2015).
- 745 64. DeFelice J, Kambic R, DeFelice R, By Its Fruit — Light Elimination Therapy for the
746 Treatment of Infertility (LET). *Its Fruit* (2014), (available at
747 <https://byitsfruit.org/research/light-elimination-therapy-for-the-treatment-of-infertility-let>).
- 748 65. K. S. Ruth, R. N. Beaumont, J. Tyrrell, S. E. Jones, M. A. Tuke, H. Yaghootkar, A. R.
749 Wood, R. M. Freathy, M. N. Weedon, T. M. Frayling, A. Murray, Genetic evidence that
750 lower circulating FSH levels lengthen menstrual cycle, increase age at menopause and
751 impact female reproductive health. *Hum. Reprod. Oxf. Engl.* **31**, 473–481 (2016).
- 752 66. A. K. Wesselink, L. A. Wise, E. E. Hatch, K. J. Rothman, E. M. Mikkelsen, J. B. Stanford,
753 C. J. McKinnon, S. Mahalingaiah, Menstrual cycle characteristics and fecundability in a
754 North American preconception cohort. *Ann. Epidemiol.* **26**, 482–487.e1 (2016).
- 755 67. L. Miolo, B. Colombo, J. Marshall, A data base for biometrics research on changes in basal
756 body temperature in the menstrual cycle. *Statistica.* **53**, 563–572 (1993).
- 757 68. B. Colombo, G. Masarotto, Daily fecundability: first results from a new data base. *Demogr.*
758 *Res.* **3**, art 5 (2000).
- 759 69. B. Colombo, A. Mion, K. Passarin, B. Scarpa, Cervical mucus symptom and daily
760 fecundability: first results from a new database. *Stat. Methods Med. Res.* **15**, 161–180
761 (2006).
- 762 70. V. Jennings, I. Sinai, Further analysis of the theoretical effectiveness of the TwoDay method
763 of family planning. *Contraception.* **64**, 149–153 (2001).
- 764 71. J. C. Barrett, J. Marshall, The risk of conception on different days of the menstrual cycle.
765 *Popul. Stud.* **23**, 455–461 (1969).
- 766 72. F. Bassi, B. Scarpa, Assessing heterogeneity in menstrual cycles by means of a multilevel
767 latent class approach. *Biodemography Soc. Biol.* **65**, 41–56 (2020).
- 768 73. P. Bortot, G. Masarotto, B. Scarpa, Sequential predictions of menstrual cycle lengths.
769 *Biostat. Oxf. Engl.* **11**, 741–755 (2010).
- 770 74. D. B. Dunson, B. Colombo, D. D. Baird, Changes with age in the level and duration of
771 fertility in the menstrual cycle. *Hum. Reprod. Oxf. Engl.* **17**, 1399–1403 (2002).
- 772 75. J. L. Bigelow, D. B. Dunson, J. B. Stanford, R. Ecochard, C. Gnoth, B. Colombo, Mucus
773 observations in the fertile window: a better predictor of conception than timing of
774 intercourse. *Hum. Reprod. Oxf. Engl.* **19**, 889–892 (2004).
- 775 76. F. Bassi, B. Scarpa, Latent class modeling of markers of day-specific fertility. *METRON.* **73**,
776 263–276 (2015).
- 777 77. F. C. Billari, A. Rosina, The effect of age on time until ovulation in female menstrual cycles:
778 A longitudinal data analysis based on mixed models. *Genus.* **57**, 71–87 (2001).
- 779 78. D. B. Dunson, D. D. Baird, B. Colombo, Increased infertility with age in men and women.
780 *Obstet. Gynecol.* **103**, 51–56 (2004).
- 781 79. E. A. Nadaraya, On Estimating Regression. *Theory Probab. Its Appl.* **9**, 141–142 (1964).
- 782 80. L. J. Christiano, T. J. Fitzgerald, The Band Pass Filter. *Int. Econ. Rev.* **44**, 435–465 (2003).
- 783 81. Time Series Analysis: Forecasting and Control, 4th Edition | Wiley. *Wiley.com* (2023),
784 (available at [https://www.wiley.com/en-](https://www.wiley.com/en-dk/Time+Series+Analysis%3A+Forecasting+and+Control%2C+4th+Edition-p-9781118619193)
785 [dk/Time+Series+Analysis%3A+Forecasting+and+Control%2C+4th+Edition-p-](https://www.wiley.com/en-dk/Time+Series+Analysis%3A+Forecasting+and+Control%2C+4th+Edition-p-9781118619193)
786 [9781118619193](https://www.wiley.com/en-dk/Time+Series+Analysis%3A+Forecasting+and+Control%2C+4th+Edition-p-9781118619193)).
- 787 82. Rubinstein, *Simulation and the Monte Carlo Method, 3rd Edition* (Wiley, Hoboken, New
788 Jersey, 3e édition., 2016).
- 789 83. D. A. Follmann, M. A. Proschan, A simple permutation-type method for testing circular

790 uniformity with correlated angular measurements. *Biometrics*. **55**, 782–791 (1999).
791 84. Lunar phase. *Wikipedia* (2023), (available at
792 https://en.wikipedia.org/w/index.php?title=Lunar_phase&oldid=1136029614).

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803 present in the paper and/or the Supplementary Materials. Original datasets are available at
804 <https://www.stat.unipd.it/ricerca/basi-di-dati> (European dataset),
805 https://epublications.marquette.edu/data_nfp/7/ (Marquette dataset),
806 <https://doi.org/10.7278/S50d-4gxs-s4hj> (Utah dataset).

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809 **Figure Legends**

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812 **Fig. 1. Composition of the European and North American datasets.** Details are given in
813 Table 1.

814

815 **Fig. 2. Distribution of menstrual cycle lengths in the European (left) and North American**
816 **(right) datasets.**

817

818 **Fig. 3. Examples of typical actograms for three European women.** (A) Overall long-term
819 stability with occasional jumps; (B) overall long-term stability with superimposed oscillations
820 lasting less than a year; (C) overall stability with rapid random fluctuations. The x-axis
821 represents the duration of each menstrual cycle, the y-axis the number of consecutive menstrual
822 cycles. The length of each blue line represents the duration of individual menstrual cycles; each
823 new cycle is shown below the previous one. For each woman, the mean cycle duration was used
824 as an estimate of the infradian period of her menstrual cycle, and the actogram was plotted with a
825 normalized time axis (modulo the infradian period). This makes it possible to visually follow
826 how the duration of each menstrual cycle compares to the average cycle duration (black line),
827 and how it varies over time. The reference time is therefore the average period of each woman's
828 menstrual cycle.

829

830 **Fig. 4. Examples of typical actograms of three European women, with oscillations**
831 **estimated by smoothing.** (A) Overall long-term stability with occasional jumps; (B) overall
832 long-term stability with superimposed oscillations lasting less than a year; (C) overall stability
833 with rapid random fluctuations. The brown line shows the result of the smoothing using a long
834 time period of the order of one year (12 cycles). The orange line shows the result of the fine
835 Christiano-Fitzgerald smoothing, which emphasizes the oscillations.

836

837 **Fig. 5. Correlations between the lengths of successive cycles (European dataset).** The
838 analysis is performed using the autocorrelation function (ACF, left) and the partial
839 autocorrelation function (PACF, right). Each gray line is the average of the correlations obtained
840 for each woman on randomly swapped data (permutation). The red line is the average of the
841 correlations obtained on real non-swapped data. * Significant correlations are indicated by
842 asterisks (bilateral test, with Bonferroni correction).

843

844 **Fig. 6. Correlations between the lengths of consecutive cycles (North American dataset).**
845 Analysis is performed using the autocorrelation function (ACF, left), and the partial
846 autocorrelation function (PACF, right). Each grey line is the average of the correlations obtained
847 for each woman on randomly swapped data. The red line is the average of the correlations
848 obtained on real un-swapped data. * indicates a significant correlation (bilateral test, with
849 Bonferroni correction).

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851 **Fig. 7. Menstrual day and moon phase (European dataset).** On the left (A), the analysis
852 includes all the data (26,912 cycles of 2,303 women). On the right (B), the analysis focuses on
853 the cycles of women whose average cycles are in the range [28-30] (9,385 cycles of 775
854 women). The green dots represent the number of menstrual cycles (first-day, see Table 3). The
855 green line is the result of circular smoothing. The blue + sign indicates the significance on the

856 bilateral test (Monte-Carlo test with 5,000 iterations), without Bonferroni correction. The circular
857 X-scale corresponds to the phase of the moon (clockwise: new moon [3 o'clock], waxing
858 crescent, first quarter, waxing gibbous, full, waning gibbous, last quarter, waning crescent). The
859 Y-scale corresponds to the frequency of occurrence of the first menstrual day as a function of the
860 moon phase; the lowest frequency observed is in the center (respectively 3,252 on the left and
861 1,189 on the right), the highest is at the outermost edge (respectively 3,533 on the left and 1,440
862 on the right).

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864 **Fig. 8. Menstrual day and lunar phase – North American dataset**

865 On the left (A), the analysis includes all data for which menstruation start date was available
866 (3,137 cycles from 562 women). On the right (B), the analysis focuses on the cycles of women
867 whose average cycles are in the range [28-30] days (1,611 cycles from 222 women). The green
868 dots represent the number of menstrual cycles that occurred at given lunar phases (first-day, see
869 Table 3). The green line is the result of circular smoothing. The blue + sign indicates the
870 significance on the bilateral test (Monte-Carlo test with 5,000 iterations), without Bonferroni
871 correction. The circular X-scale corresponds to the phase of the moon (clockwise: new moon [3
872 o'clock], waxing crescent, first quarter, waxing gibbous, full, waning gibbous, last quarter,
873 waning crescent). The Y-scale corresponds to the frequency of occurrence of the first menstrual
874 day as a function of the moon phase; the lowest frequency observed is in the center (respectively
875 356 on the left and 105 on the right), the highest is at the outermost edge (respectively 431 on the
876 left and 197 on the right).

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Tables

Table 1. Datasets for each analysis.

Analysis	Selection criteria	Dataset	European cycles (women)	North American cycles (women)
		London	16,060 (1,057)	
		Fertili	5,199 (619)	
		Billings	1,970 (178)	
		Vicenza	1,911 (256)	
		Barrett-Marshall	1,772 (193)	
		Utah		3,137 (562)
		Marquette		1,649 (159)
			Total : 26,912 (2303)	Total : 4,786 (721)
Actogram and description of oscillations	Series of 18 menstrual cycles or more		12,728 (425)	331 (12)
Correlation of the durations of successive cycles	Series of 13 menstrual cycles or more		16,568 (685)	-
	Series of 7 menstrual cycles or more		-	3,529 (314)
Menstrual day and moon phase	Cycles whose first day date and duration are known		26,912 (2303)	3,137 (562)

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Table 2. Statistical significance of the correlation of the durations of successive cycles.

Dataset	cycles (women)	model	Lag*					
			1	2	3	4	5	6
European	16,569 (685)	ACF	0.9480	<u><0.0001</u>	<u>0.0004</u>	<u>0.0024</u>	0.8306	0.0342
North American	3,529 (314)	ACF	0.3482	0.4354	<u>0.0084</u>	0.5582	0.5504	0.8718
European	16,569 (685)	PACF	0.9490	<u>0.0032</u>	<u><0.000</u>	<u>0.0010</u>	0.4268	0.1742
North American	3,529 (314)	PACF	0.3626	0.6072	<u>0.0030</u>	0.5806	0.0114	0.9364

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* Consider the discrete sequence of menstrual cycle lengths. For lag 1, we compare the length of the menstrual cycle with the previous one. We calculate the correlation of the length of the menstrual cycle with the previous one. For lag 2, we calculate correlation with the second to last one. Etc. This table provides the p-value. A significant p-value indicates that the correlation is not compatible with simple chance. ACF : autocorrelation function, PACF: partial autocorrelation function

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Table 3. Lunar phase of menstruation onset - Chi-square (χ^2) and Rayleigh test.

	Subset	New	Waxing crescent	First quarter	Waxing gibbous	Full	Waning gibbous	Last quarter	Waning crescent	χ^2 p value	Rayleigh Test per cycle p value	Rayleigh Test per woman p value
European	Total	3,362*	<u>3,533</u>	3,423	3,354	3,393	3,319	3,276	3,252	0.0214	0.0056	0.0986
North American	Total	356	367	382	422	<u>431</u>	382	407	390	0.0952	0.0226	0.0927
European	[28-30]	1,321	<u>1,440</u>	1,379	1,284	1,273	1,259	1,198	1,189	<0.0001	<0.0001	0.0090
North American	[28-30]	105	114	127	165	<u>197</u>	136	154	152	<0.0001	<0.0001	0.0313

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* number of menstrual cycles

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Table 4. Lunar phase of menstruation onset - Monte-Carlo test (5,000 iterations).

		Place of the observed value in the distribution of simulated values*							
	Subset	New	Waxing crescent	First quarter	Waxing gibbous	Full	Waning gibbous	Last quarter	Waning crescent
European	Total	0.4828*	<u>0.9888</u>	0.7858	0.4180	0.6866	0.2592	0.1054	0.0726
North American	Total	0.0640	0.1390	0.3362	0.8778	<u>0.9600</u>	0.3468	0.6946	0.4454
European	[28-30]	0.6724	<u>0.9930</u>	0.9258	0.4334	0.3796	0.2944	0.0488	0.0372
North American	[28-30]	0.0158	0.0400	0.1770	0.8684	<u>0.9964</u>	0.3410	0.6954	0.6618

899 * the observed values are shown in Table 3

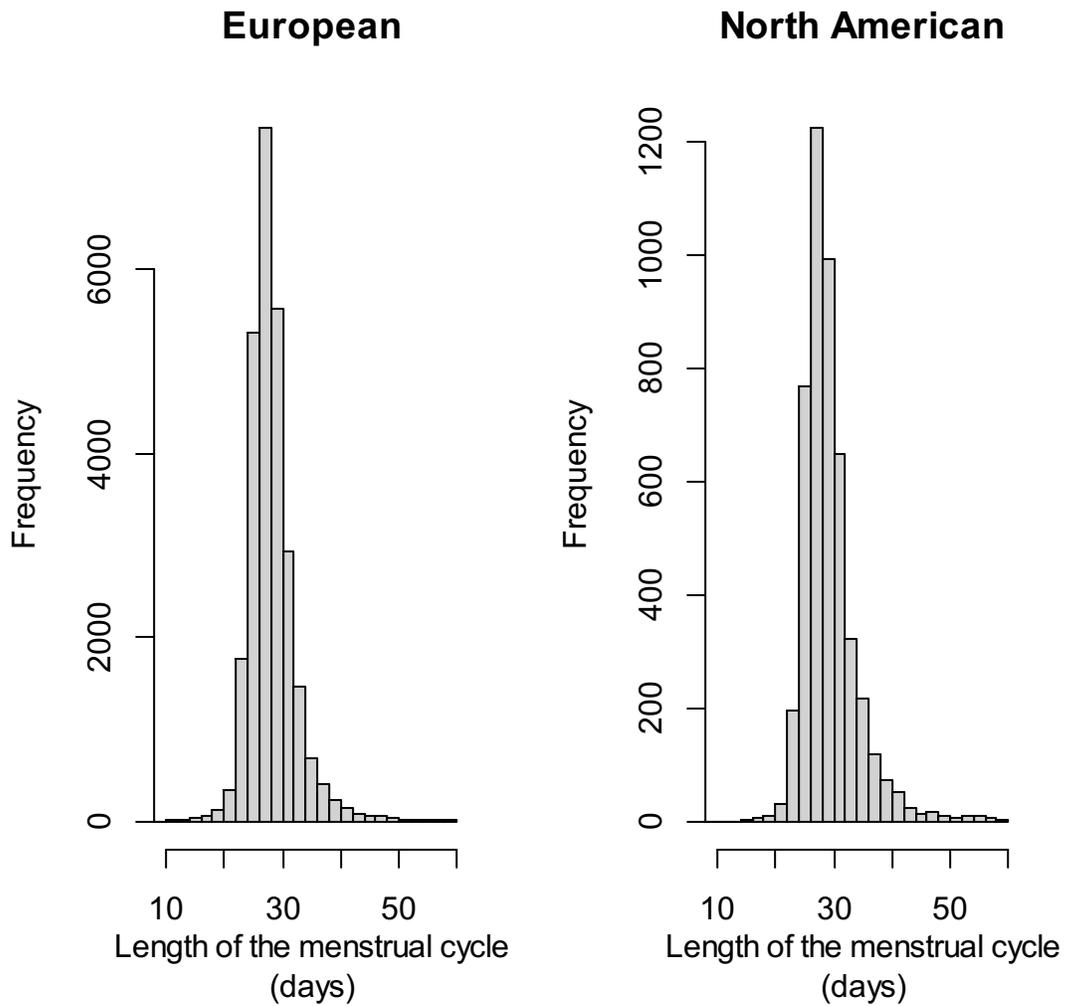


Fig. 2. Distribution of menstrual cycle lengths in the European (left) and North American (right) datasets.

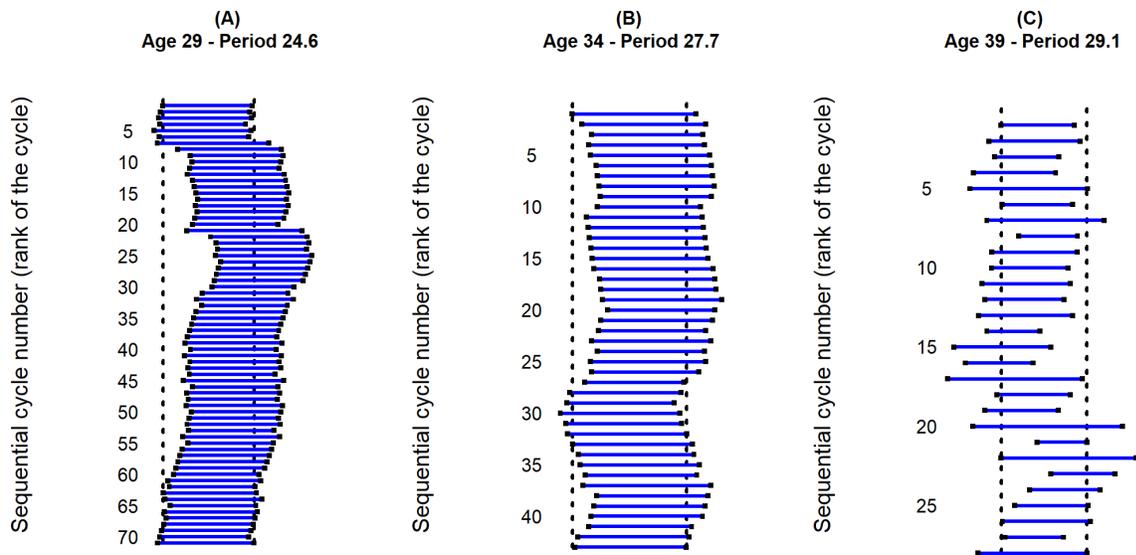


Fig. 3. Examples of typical actograms for three European women.

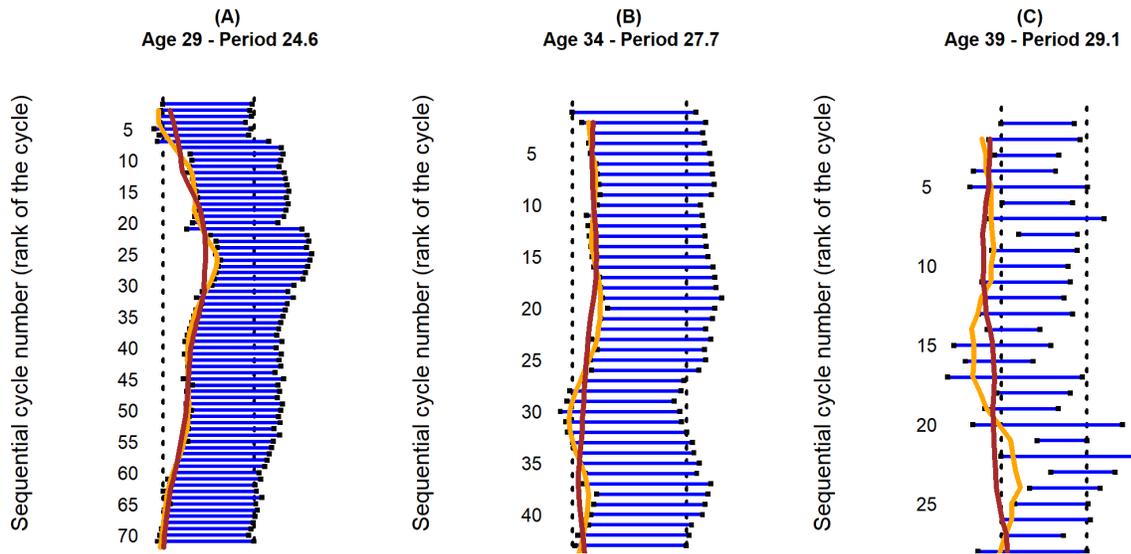


Fig. 4. Examples of typical actograms of three European women, with oscillations estimated by smoothing.

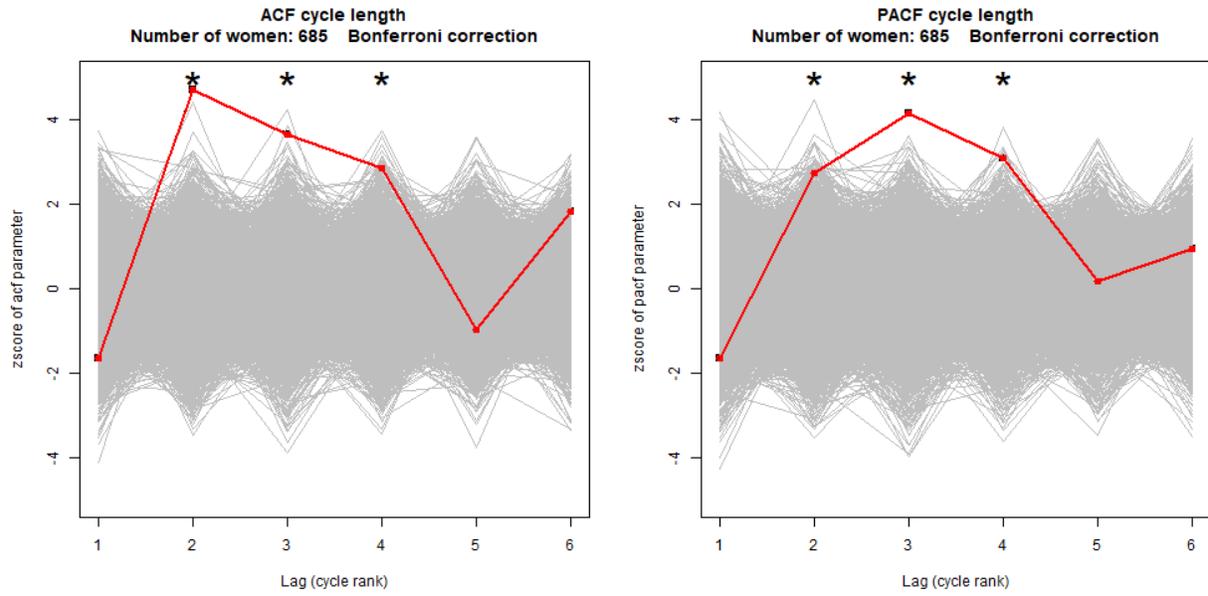


Fig. 5. Correlations between the lengths of successive cycles (European dataset).

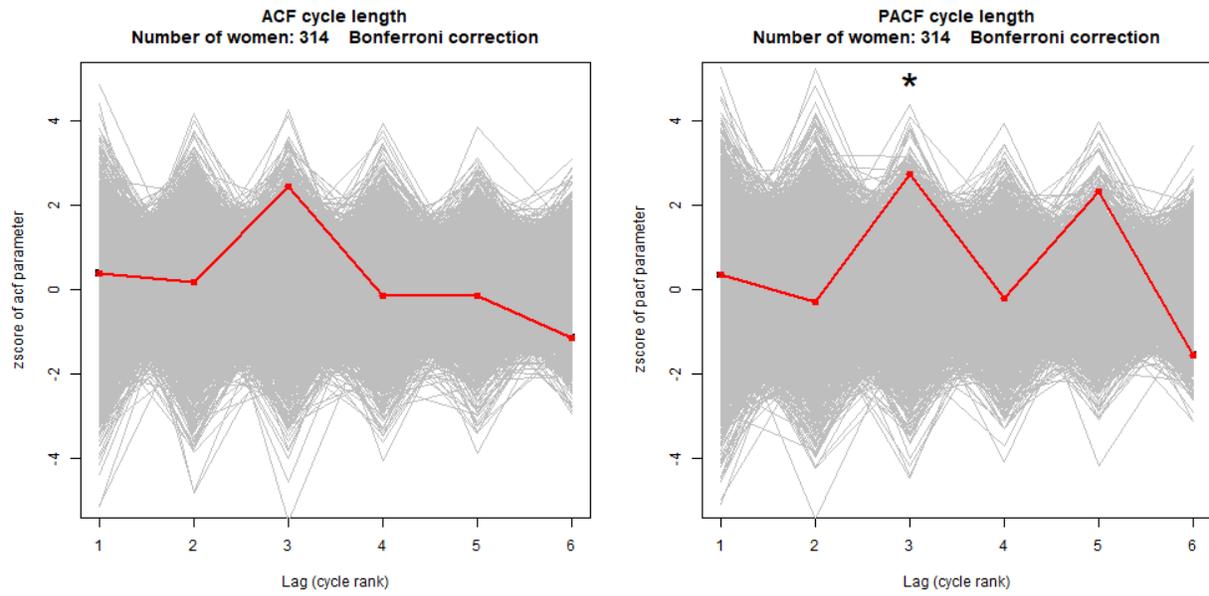


Fig. 6. Correlations between the lengths of consecutive cycles (North American dataset).

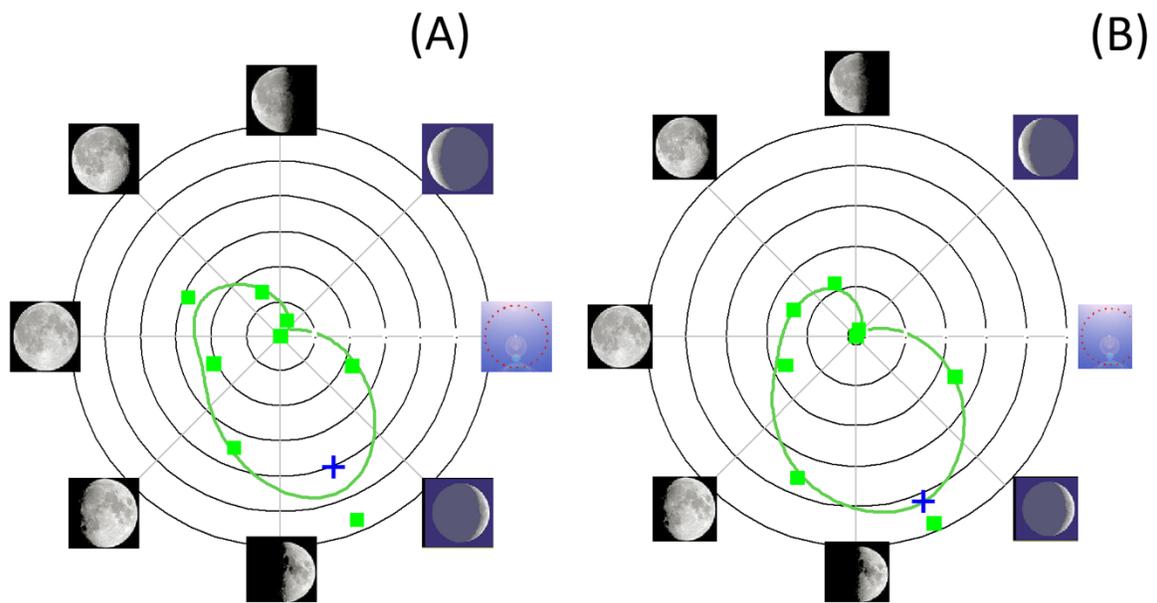


Fig. 7. Menstrual day and moon phase (European dataset).

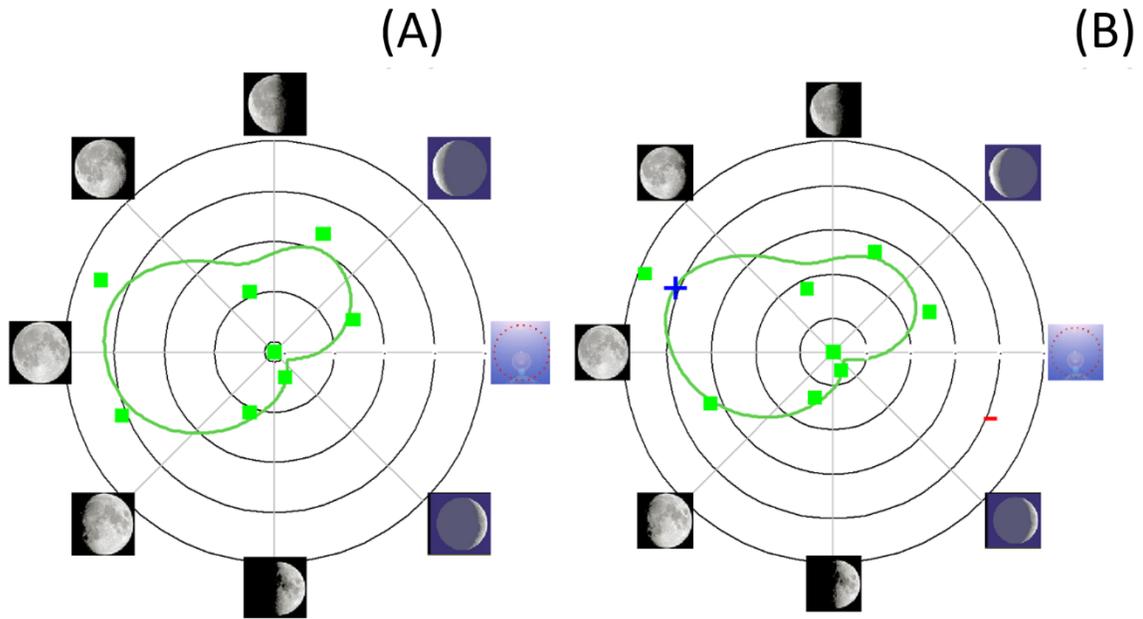


Fig. 8. Menstrual day and lunar phase – North American dataset