Influence of the Menstrual Cycle on the Performance of Different Physical Tests Specific to Handball

MOUSSOUAMI Simplice Innocent1,2*, AGBODJOGBE Kpedetin Wilfrid Dieu Donné2, BOUSSANA Alain Marc2, LOUNIONGO God2, ALONGO Yvon Rock Ghislain2 and BIO NIGAN Issiako1

1Sport, Health and Evaluation Research Unit (UR/SSE), National Institute for Youth, Physical Education and Sport (INJEPS), University of Abomey-Calavi (UAC), Benin
2Laboratory of Physiology of Effort and Biomechanics (LPB), Higher Institute of Physical Education and Sport (ISEPS), University Marien Ngouabi, Congo
3Laboratory of Physiology of Effort, National Institute for Youth, Physical Education and Sport (INJEPS), University of Abomey-Calavi (UAC), Benin

*Corresponding author: Simplice Innocent Moussouami, Sport, Health and Evaluation Research Unit (UR/SSE), National Institute for Youth, Physical Education and Sport (INJEPS), University of Abomey-Calavi (UAC), 01 BP: 169, Porto-Novo, Benin, Tel: 00242-06-636-66-97/00229-90-23-67-60

Abstract

Nowadays, several scientific studies focus women’s the menstrual cycle and its impact on sports performance. However, the results of these studies are controversial. In this regard, the aim of this study is to evaluate the effects of the menstrual cycle on physiological responses, intermittent Yo-Yo test performance, repeated sprints and medicine-ball in elite women handball players. The sample of this study is 18.71 ± 0.72 years older female players. The following handball specific physical tests were performed: intermittent Yo-Yo test, repeated sprints and medicine-ball whose lactate concentration was also measured. Endurance capacity ($\dot{V}O_{2\max}$) was lower in the luteal phase (p < 0.001). In contrast, medicine-ball performance, and repeated sprinting ability are better in the Follicular Phase (FP). The menstrual cycle affected performance on the Yo-Yo IET, exercise HRmax and muscle strength. Better performance is observed during the follicular phase.

Keywords

Performance, Menstrual cycle, Sport, Congo

Introduction

Handball is a team sport characterized by intense and intermittent efforts, it involves, among other things, acceleration, changes of direction, sprinting, jumping, shooting, slow running, walking, dueling with wrestling-like paces [1,2]. In its current form, a handball match lasts 60 minutes, consisting of two halves of 30 minutes each separated by a 10-minute break. Intense efforts during the match are characterized by a heart rate above 85% of maximum [3], a maximum oxygen consumption ($\dot{V}O_{2\max}$) of up to 61 ml.kg$^{-1}$.min$^{-1}$ in men and 52 ml.kg$^{-1}$.min$^{-1}$ in women [2]. Recent changes in handball rules make the game much faster, with less time loss between phases [4] and require players to make maximum physical efforts during matches. To achieve the best performance, teams must, therefore, through consistent physical preparation meet these physical and physiological demands [5]. To this end, several physiological, environmental and hormonal parameters during a match have to be taken into account. These parameters are dependent on several factors including gender, circulating blood volume, heart rate, serum lactic acid level, body temperature, scapular limb strength, pelvic limb power and maximum oxygen consumption ($\dot{V}O_{2\max}$) which are important physiological characteristics for optimal handball performance. The rules of the game, the training methods are identical for both sexes. But in the...
training process, the menstrual cycle in women, must be an important factor that must be taken into account.

The menstrual cycle is mainly divided into two phases: a follicular phase, which begins with the bleeding phase, and a luteal phase. These phases of the menstrual cycle are associated with hormonal fluctuations and can affect the physiology of the woman’s body. Furthermore, it is assumed that the menstrual cycle affects women’s physiological indices when compared in the luteal and follicular phases. Indeed, estrogen and progesterone receptors have been identified in skeletal muscle. As skeletal muscles are organs that play a very important role in movement and in HB racing, cyclical fluctuations of these hormones can directly influence training results.

Recently several studies have reported that women with a normal menstrual cycle may be more prone to decreased performance and post-exercise muscle soreness in the early follicular phase. Shahidi, et al. [6] showed that some physiological parameters such as $\bar{VO}_2$ max and body temperature are influenced by menstrual cycles. In contrast, Guler, et al. [7] concluded that menstrual cycles in female futsal players do not affect flexibility, power, speed, anaerobic and aerobic performance. Blanca Romero-Moraleda, et al. [8] report that resistance-trained eumenorrhoeic women have similar speed, strength and power performance when exercising with loads equivalent to 20, 40, 60 and 80% of 1RM during the Smith machine half-exercise during exercise in different phases of the menstrual cycle.

The effects of the menstrual cycle on body composition, physical capacity and physiological parameters during and after exercise have been studied in the literature, but the results remain incomplete and contradictory. Further studies on this topic are therefore needed. Furthermore, the literature lacks studies on the relationship between the different phases of the menstrual cycle and physical performance in female handball players. Therefore, the aim of this study is to evaluate the effects of the menstrual cycle on physiological responses and performance to repeated sprints in elite women handball players.

**Material & Methods**

**Participants**

A total of 43 elite female players in the junior and senior age group in Brazzaville with a normal menstrual cycle (between 25 and 28 days) took part in this research. The sample for this study was selected by reasoned choice and consisted of 22 players in the luteal phase and 21 in the follicular phase. These athletes are selected for their experience in national and international competitions. To be included in the sample for this research, each player had to meet the following inclusion criteria: be licensed during the 2018 season. In addition to the general criteria, athletes had to participate in at least one national champion and had never used oral contraceptives. The purpose and risks of this study are explained and each participant signed a written consent. The study was validated by the ethics committee of the Higher Institute of Physical Education and Sport in accordance with the Helsinki recommendations.

**Data collection protocol**

Before the start of the experiment and the beginning of each phase of the menstrual cycle, anthropometric, physiological, urine and blood samples are taken. Heart rate, core temperature and serum lactate levels are also measured. Urine collection was used to measure urine density to determine water status. The heat stress index is calculated. During this study, field tests such as the Yo-Yo IRT2 (9) and medicine-ball are performed for the respective calculation of $\bar{VO}_2$ max and scapular limb strength. Subjects are also subjected to a capacity test of repeated sprints. These tests are used on day 6 and 10 of the follicular phase and day 20 and 24 of the luteal phase (10). All measurements are repeated at the end of each phase, at the same times of day. Subjects continued training during the follicular (FP) and luteal (LP) phases except on the days of the experiment. They are forbidden to drink caffeine, alcohol or any other drug.

**Measurements and tests**

**Anthropometric measurements**

**Height:** The equipment used to measure height is a two-metre-high ZT-150A toise (Perlong Medical with Equipment, China). The height measurement is consisted of determining the stature of the subjects. It determines the structure measured from the foot to the top of the skull. The subject is asked to stand barefoot on a flat, compact surface in an upright position (arms at the side of the body, heels together, head upright, looking towards the horizon). The reference point being the feet and the vertex (top of the head), the researcher placed the graduated scale behind the subject, and compressed the subject’s hair using a square (point) fixed to the scale, graduated to the nearest centimeter.

**Body mass:** A digital scale of the brand Personnal Scale (model: 2003A; max weight: 180 kg, d = 0.1 kg) is used to measure weight. The weight measurement consisted of determining the subjects’ body mass. The subject stood on the scale platform, upright with his or her hands at his or her sides, staring straight ahead and the body mass value was measured in kilograms from the reading on the scale. Height was measured using a stadiometer (to the nearest 1 cm) with a participant standing upright against a vertical surface, and weight was measured using a weighing machine to the nearest 1 kg with subjects standing still, with their feet approximately 15 cm apart and the weight evenly distributed on each leg. Participants were instructed to
wear light clothing (culturally appropriate) and not to wear shoes when measuring body weight. BMI in kg/m² was calculated by the Quetelet index.

**Biometric measurements:** Core body temperature: The equipment used to determine rectal temperature (Tr) was MT 101R automatic thermometers (Hangzhou Sejoy, China) for personal and individual use. Before and after each use, the administrator cleaned the probe with alcohol-soaked cotton wool for disinfection. The researcher switches on the thermometer and this will cause a beep to be heard indicating that the device is actually working. A display test is then performed and the last measurement in memory is displayed. This was measured in the rectum using a Biopac MP100 system with a 1.7 mm diameter SKT100C transducer module. To insert this system into the rectum we wrapped the thermistor probe in a plastic film lubricated with gel before inserting it.

Heart rate: A Geonote heart rate monitor (Decathlon, France) is used to measure heart rate. The subject wore a belt with heart activity sensors which were transmitted to a watch worn on the wrist. The subject is instructed to turn on the heart rate monitor and then press the “effort indicator” button until the heart appeared, marking the heart activity. After a few minutes of rest, the administrator, having noted the stability of the heart rate value, took and recorded this value. This measurement is consistent of determining the heart rate in its value.

**Samples:** Serum lactic acid level: The equipment used to measure the serum lactic acid level is the lactate pro II. This measurement is consistent of the determination of lactic acid in blood. The blood is taken from the earlobe by a needle and collected on a slide for examination.

Water status: The equipment used to determine water status is the PEN-Urine clinical refractometer, brand name Atago (Japan). The water status is measured from the urine density. Thus, an athlete is considered normo-hydrated when his urine density (UD) < 1.020. He was dehydrated when 1.020 < UD < 1.030. The athlete was severely dehydrated when the UD > 1.030.

**Menstrual cycle information:** The materials used to obtain information about the participants’ menstrual cycle were the mobile application mycalender, period-Tracker, US and a menstrual diary. The length of the menstrual cycle and the start of each phase were determined using a mobile application (Mycalendar®, Period-Tracker, US) and a menstrual diary indicating the date of menstruation, the duration of menstruation and the discomfort experienced in the days before and during menstruation. Participants collected these data for a complete menstrual cycle, starting with the randomly allocated phase.

**Physical effort tests**

Repeated sprint capacity: This test is made of six sprints at maximum speed interspersed with 10s of recovery. This test consists a assessing an individual’s ability to repeat effort intervals over a prolonged period. On a flat surface the researcher measured a distance of 35 m where he placed two image sensors at the beginning and end of the journey. Using a stopwatch, the researcher starts a 35 m shuttle run at maximum speed.

To assess the CSR or anaerobic sprinting in running, the 6 × 35 m test of was used. The test consists of six repeated 35m sprints at full speed with a 10 second recovery time between each sprint. The time performance will be used to calculate the power of each sprint, as follows:

**Yo-yo Intermittent Recovery test (Yo-yo IRT2):**

Yo-yo Intermittent Recovery test (Yo-yo IRT2) was used to calculate $\dot{V}O_{2max}$ [9,10]. This test is based on an intermittent shuttle run performed over a distance of 20m with an active recovery zone of 5 m. At the signal of the audio recording that serves as a guide to the test, the athlete runs the distance back and forth and then pauses for 10s in active recovery. From the level reached by the athlete, the distance covered was determined and the $\dot{V}O_{2max}$ was calculated according to the following equation:

$$\text{Yo-Yo IRT2: } \dot{V}O_{2max} (\text{mL/min/kg}) = IRT2 \text{ distance (m) } \times 0.0136 + 45.3$$

**Medicine-ball eccentric throw test:** This test is a valid tool to measure the strength and power of the scapular limbs. Using a 2 kg ball, the subject throws it as far as possible with a flexion-extension movement of the arms above the head. The subject maintains a standing posture for the throws, and must not bend his or her legs, arch backwards, or bend the torso over the legs. After three trials with a 60-second break in between, the examiner selects the best of the three for analysis.

**Statistical analysis**

The data from this study were processed with Statistica software (Stat. Soft. Inc., version 12.0). The normality of the distribution of the variables was checked with the Kolmogorov Smirnov test and the homogeneity of variances was tested with the Levene test. The two-factor ANOVA (Time x cycle periods) are used to test for interactions between measurement times and groups. The Wilcoxon rank test was used to test the measurement time effect in each period. Comparisons between the two periods of the cycle were made with the Man Whitney U test. The significance level of the statistical tests was set at p < 0.05.

**Results**

Players in the PF and PL groups were 18.36 ± 0.84 and 18.71 ± 0.72 years-old, respectively. Height, body mass and BMI were similar in both groups (p > 0.05). HR recorded in the PF group was on average significantly
differences were found between the two groups at the end of the program (Table 2).

Table 1: Anthropometric and physiological characteristics of the players.

<table>
<thead>
<tr>
<th></th>
<th>Effectif total (n = 43)</th>
<th>FP Group (n = 22)</th>
<th>LP Group (n = 21)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.53 ± 0.79</td>
<td>18.36 ± 0.84</td>
<td>18.71 ± 0.72</td>
<td>1.15</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.80 ± 4.60</td>
<td>160.61 ± 5.41</td>
<td>165.09 ± 3.73</td>
<td>0.09</td>
</tr>
<tr>
<td>BM (kg)</td>
<td>58.48 ± 7.12</td>
<td>59.88 ± 8.09</td>
<td>57.01 ± 5.76</td>
<td>0.13</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.33 ± 2.87</td>
<td>21.61 ± 3.00</td>
<td>21.03 ± 2.77</td>
<td>0.72</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>85 ± 6</td>
<td>83 ± 4</td>
<td>87 ± 5**</td>
<td>0.004</td>
</tr>
<tr>
<td>Trec (°C)</td>
<td>37.57 ± 0.58</td>
<td>37.26 ± 0.50</td>
<td>37.90 ± 0.48**</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The numbers in the boxes represent the means ± standard deviations (Mean ± SD); FP: Follicular Phase; LP: Luteal Phase; BM: Body Mass; BMI: Body Mass Index; HR: resting heart rate; Trec: core temperature; **: difference between FP group and LP group, significant at p < 0.01; ***: difference between FP group and LP group, significant at p < 0.001

Table 2: Variation in anthropometric parameters of female players (n = 43).

<table>
<thead>
<tr>
<th></th>
<th>PF Group (n = 22)</th>
<th>After</th>
<th>LP Group (n = 21)</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (kg)</td>
<td>59.88 ± 8.09</td>
<td>59.33 ± 8.03**</td>
<td>57.01 ± 5.76</td>
<td>56.61 ± 5.59***</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.61 ± 3.00</td>
<td>21.40 ± 2.94***</td>
<td>21.03 ± 2.77</td>
<td>20.83 ± 2.46***</td>
</tr>
</tbody>
</table>

Numbers in boxes represent means ± standard deviations (Mean ± SD); FP: Follicular Phase; LP: Luteal Phase; : difference between FP group and LP group, significant at p < 0.05; ***: difference between FP group and LP group, significant at p < 0.001

Table 3: Assessment of physiological parameters and performance during the different phases of the menstrual cycle in the women handballers studied.

<table>
<thead>
<tr>
<th></th>
<th>FP Group (n = 22)</th>
<th>LP Group (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRre (bpm)</td>
<td>79.3 ± 3.4</td>
<td>85.7 ± 4.6</td>
</tr>
<tr>
<td>HRmax théo (bpm)</td>
<td>202 ± 1</td>
<td>202 ± 1</td>
</tr>
<tr>
<td>HRmax (bpm)</td>
<td>161 ± 3</td>
<td>174 ± 5</td>
</tr>
<tr>
<td>Trec (°C)</td>
<td>38.81 ± 0.28</td>
<td>38.85 ± 0.51</td>
</tr>
<tr>
<td>Densité Urinaire (DU)</td>
<td>1.01 ± 0.008</td>
<td>1.01 ± 0.10</td>
</tr>
<tr>
<td>% PP (%)</td>
<td>1.30 ± 0.45</td>
<td>1.73 ± 0.62</td>
</tr>
<tr>
<td>IPCT</td>
<td>6.48 ± 0.56</td>
<td>6.44 ± 1.01</td>
</tr>
<tr>
<td>(\overline{V}O_{2\max}) (mL/min/kg)</td>
<td>49.28 ± 1.07**</td>
<td>47.40 ± 0.76</td>
</tr>
<tr>
<td>PMB</td>
<td>7.28 ± 0.35**</td>
<td>6.73 ± 1.01</td>
</tr>
<tr>
<td>CSR (min)</td>
<td>6.81 ± 0.38</td>
<td>7.01 ± 0.32</td>
</tr>
<tr>
<td>Pmoy</td>
<td>240.87 ± 58.30'</td>
<td>209.47 ± 25.81</td>
</tr>
<tr>
<td>Pmax</td>
<td>308.02 ± 68.63'</td>
<td>269.34 ± 16.09</td>
</tr>
<tr>
<td>IF</td>
<td>40.88 ± 7.23</td>
<td>40.44 ± 12.57</td>
</tr>
</tbody>
</table>

Numbers in boxes represent mean values ± standard deviations; HRre: exercise heart rate; HRmax théo: maximum heart rate; Trec: core temperature; % PP: percentage weight loss; IPCT: heat stress index; \(\overline{V}O_{2\max}\): maximal oxygen consumption; PMB: medicineball performance; CSR: repeated sprint capacity; Pmoy: mean power; Pmax: maximum power; : difference between FP group and PL group, significant at p < 0.05; *: difference between FP group and PL group, significant at p < 0.01; **: difference between FP group and PL group, significant at p < 0.001

higher than that recorded in the PL group (83 ± 4 versus 87 ± 5 bpm; p = 0.004). Core temperature was significantly lower (37.26 ± 0.5 °C; p < 0.001) in the FP group than in the PL group (Table 1).

The body parameters recorded at the beginning of the repeated sprints were identical between the PF and PL groups. However, significant changes were noted in both groups after the repeated sprints. But no significant differences were found between the two groups at the end of the program (Table 2).

Table 3 shows the variation of physiological parameters and performance during the different phases of the menstrual cycle in female handball players. In the Luteal Phase (LP) the players had a higher exercise heart rate compared to the follicular phase (174 ± 5 bpm versus 161 ± 3 bpm; p < 0.001). Consequently,
they achieved significantly lower endurance capacity performance ($\dot{V}O_{2\text{max}}$) (47.40 ± 0.76 mL/min/kg versus 49.28 ± 1.07 mL/min/kg; p < 0.001). In contrast, the best medicine-ball performance, repeated sprint ability was recorded in the follicular phase compared to the luteal phase.

During repeated sprints, [La] increased by after post-exercise [La] increased but is significantly lower than that observed in the follicular phase (7.28 ± 0.35 mmol/L versus 6.73 ± 1.01 mmol/L; p < 0.001 and 308.02 ± 68.63 mmol/L versus 269.34 ± 16.09 mmol/L; p = 0.02) (Figure 1).

**Discussion**

The aim of the study is to determine whether each phase of the menstrual cycle had an influence on the physical performance of women handball players. The main findings of the study are: 1) Female handball players performed better in the Yo-Yo IET test, and muscle strength. 2) Exercise HRmax was affected in the luteal phase.

Available data on the effect of the menstrual cycle on performance are contradictory in nature. Although many studies have shown that the menstrual cycle has an influence on physical performance [11], a decent number of studies have concluded that there is no variation in performance between periods of the menstrual cycle [12]. In the literature, and to our knowledge, this is the first study to evaluate the effects of menstrual cycle phase on the performance of different physical tests in handball. In this study, the phase of the cycle affected the endurance capabilities of the players in the Yo-Yo test. Physical performance in handball is considered to be attributed to the training status and aerobic capacity of athletes. The results of the present study are in agreement with those of Bandyopadhyay, et al. [13] who in a cross-sectional survey of 55 women with a normal cycle suggested that endurance capacity is significantly low in the follicular phase. In this investigation, VO2max is assessed directly by the Brude treadmill test method. Shahidi, et al. [6] reached the same conclusion in a study of older adolescent girls with a normal menstrual cycle of 25-35 days [14].

The most plausible explanation for the differences in endurance performance observed during the different phases of the menstrual cycle would certainly be the fluctuation in the concentration of ovarian hormones. The latter could have consequences on performance due to changes in core temperature. Indeed, body temperature is slightly higher by 0.3 to 0.5 °C during the luteal phase compared to the follicular phase [15]. Therefore, it can be postulated that elevated temperature during the luteal phase alters physiological responses during exercise. Several studies have suggested that elevated basal body temperature is associated with increased fatigue and impaired aerobic performance.

These results suggest that progesterone-induced temperature elevation may explain the variation in

![Figure 1: Changes in lactate concentration induced by repeated sprints.](image-url)
endurance performance. However, other observations suggesting an association between endurance performance and menstrual cycle phase are not consistent with this hypothesis. In addition, numerous studies have shown that endurance performance is more reduced in the luteal phase compared to the follicular phase under hot and humid conditions (32 °C, 62% relative humidity) [16,17].

Regarding lactate concentrations between the 3- and 5-minute post-exercise phases, significant differences are observed in this study. Consistent with the results of the present study, Osthuyse, et al. [12] suggested that during the luteal phase, lactate concentration was reduced due to a low demand on aerobic glycolysis during this phase. Interestingly, however, the data from this study did not indicate any difference in lactate production at rest or at peak lactate concentration. These results are in agreement with those of Vaiksaar, et al. [18], who found no difference in lactate production in the same way in female rowers completing a one-hour row at 70% of $\dot{V}O_{2\max}$. When we compare the results of different studies on the effect of the menstrual cycle on performance seems difficult due to the specificities and physical and physiological demands of each sport discipline and the tests used.

The data from this study show no significant difference in sprint performance between the follicular and luteal phases. This result is in agreement with Julian, et al. [19] who reports that repeated sprint performance showed no difference in female footballers.

Another finding of this study is the lack of effect of cycling on muscle strength in the medicine-ball throwing test. Our results are consistent with those of previous studies [17] in which the authors reported no significant differences in muscle strength between phases of the menstrual cycle. However, in these previous studies, the handgrip test and the isometric test of the knee flexors and extensors are the most commonly used to determine muscle strength in athletes. In this study, we have selected the medicine-ball throw test to determine whether the menstrual cycle affected pelvic limb muscle performance. In contrast, another study examined the effects of training during the follicular and luteal phases on muscle strength, muscle volume and microscopic parameters. The results of this study have shown that the follicular phase results in an increase in muscle strength and muscle diameter compared to the luteal phase due to the high concentrations of free testosterone during this phase [20]. These observed differences could certainly be due to alterations in estradiol and progesterone related to the menstrual cycle. Indeed, several studies have shown that ovarian hormones fluctuate during the menstrual cycle [12,21].

Some limitations of this study deserve to be mentioned and discussed. The sample size of the study makes it impossible to reliably generalize the results to the whole population of female handball players. Tracking of menstruation is used to determine the start of the menstrual cycle. Therefore, the menstrual calendar run alone does not provide all relevant information regarding the phase of the menstrual cycle [22]. Female serum sex hormones are also measured to determine the length of the cycle and the beginning/end of each cycle. No data on the exact amount of blood lost during the menstrual period is obtained. There are a few limitations to this study that are worth noting despite its strengths. This study only looked at two phases of the cycle in female handball players and female serum hormones were not measured to confirm cycle length, phase onset and phase end. The number of participants is small and does not allow for the generalization of these results to all female handball players. Studies with larger numbers are needed to validate these results. Nevertheless, athletes and coaches need to be consoled on the menstrual cycle, its relationship to performance, and the wide variability that exists between individuals. Many athletes are currently self-managing and should report menstrual concerns to both their GP and sports staff.

**Perspectives**

Further studies should consider: 1) Monitoring the evolution of physical parameters throughout the menstrual cycle and assessing the actual duration of the menstrual cycle with serum hormone measurements; 2) Analyzing the differences between the variation of these physical parameters in players with a regular cycle and those with an irregular cycle.

**Conclusion**

This study has assessed the influence of the different phases of the menstrual cycle on physiological responses, performance in the intermittent Yo-Yo test, repeated sprints and medicine-ball in elite women handball players. The results obtained suggest that the follicular phase induced a decrease in performance in the Yo-Yo IET test, muscle strength as well as the ability to perform repeated sprints. Assessing performance on different physical tests in handball will help players and coaches understand the specific adaptations of the menstrual cycle phase to exercise and sports performance.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**Authors’ Contributions**

All authors have contributed to this article, agree with the content and have approved the final manuscript.

**Acknowledgements**

The authors would like to thank all the handball players for their participation in the study and all the team leaders who believed in this study. Thanks also to the colleagues in the laboratory for their valuable contribution.
References


