Ovarian follicle growth dynamics during the postpartum period in Holstein cows and effects of contemporary cyst occurrence

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ABSTRACT: The indicators of follicle development with regard to the growth wave order, the first ovulation, animal parity, and also with regard to the simultaneous presence or absence of a follicular cyst were determined in cows in the course of 60 days postpartum. Follicular dynamics were monitored daily by ultrasonography. The animals were assigned to three groups based on the time of the 1st ovulation: G1 (n = 9) – the 1st dominant follicle (DF) ovulated, G2 (n = 10) – ovulation occurred on the 2nd or later follicular waves, and G3 (n = 5) – no ovulation occurred during the experimental period. G1 animals showed better fertility later (no cyst, less days open, P = 0.07, less hormonal treatment, P = 0.008). The rhythm of follicular wave development was generally similar in all the animals (based on emergence of the first follicular wave, the interval from emergence to deviation, and the number of all follicular waves). Nevertheless, emergence of follicular waves and deviation occurred by 0.5–0.9 day earlier in primiparous than in multiparous cows and in G1 vs. G2, or G3, respectively (in all P < 0.05). DF development was independent of parity as well as group effects, but the maximum size and growth rate (1.2 vs. 0.8 cm/day, P < 0.05) were higher in ovulatory follicles (OF) than in regressive ones (rDF). The presence of a growing cyst decreased the probability of rDF as well as OF development (P < 0.0001). The OF growth rate was faster in the milieu of a stagnating cyst than without any cyst (P < 0.04). Therefore, the development of follicles was dramatically suppressed beyond, but nor before, deviation in the milieu of a growing cyst. Cessation of the cyst growth accelerated the development of OFs. On the contrary, a cystic structure without any significant growth can persist for weeks with no effect on successful follicular development.

Keywords: cow; postpartum; ovary; follicular wave; dominant follicle; cyst

Ovarian activity plays an irreplaceable role in the chain of events leading to pregnancy and delivery. Early resumption of ovarian activity is essential for timed cow conception, and thus for achieving an economically acceptable length of the open period. Crucial events in the resumption of ovarian activity postpartum (pp) are the emergence of the 1st follicular wave (Lucy, 2007) and selection of the dominant follicle (DF) (Mihm and Austin, 2002), which can ovulate, become atretic, or develop into a cyst or non-ovulatory follicle (Savio et al., 1990a; Beam and Butler, 1997; Sakaguchi et al., 2006). Factors affecting the fate of DFs pp are closely related to the metabolic status of animals – for instance, prepartum diet (Cavestany et al., 2009), energy balance (EB) pp (Beam and Butler, 1997), but also parity (Zhang et al., 2010). The ovarian follicular dynamics in heifers and cows is well described during the oestrous cycle (Sartori et al., 2004; Šichtař et al., 2010), but there is a lack of detailed information supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. QH91270 and S grant).
about follicular development in the early pp period, especially in high-yielding dairy cows. In the context of the onset of ovarian activity pp, authors have focused mainly on indicators such as dynamics and metabolic/hormonal profiles of the first follicular wave (Kawashima et al., 2007), days of the first ovulation (Galvao et al., 2010), the first onset of luteal activity (Hayashi et al., 2008), links to uterine involution (Sheeldon and Dobson, 2004) or to the first insemination term (Hommeida et al., 2005). It has been determined that the first follicular growth wave emerges soon after delivery and is independent of the EB of the animal (Butler, 2003). Selection of its DF (> 9 mm) takes place around day 10 pp (Savio et al., 1990a). DFs from the first follicular growth wave ovulate in 30–80% by day 20 (Crowe, 2008), and the interval from parturition to the first ovulation seems to be dependent on parity (Darwash et al., 1997; Tanaka et al., 2008). In the case of successful ovulation, corpus luteum (CL) forms and subsequent luteolysis can result in reestablishment of cyclical ovarian activity (Peter et al., 2009). There is good evidence that the 1st luteal phase pp is not regular (Opsomer et al., 1998). The question arises if the subsequent follicular growth is also affected.

Information dealing with the growth dynamics characteristics of follicles on the 2nd and further follicular waves is rare and historical (Savio et al., 1990a; Kamimura et al., 1993a). Since the time of these experiments, namely the milk yield and thus metabolic status of dairy cows as well as their reproductive performance have changed. Therefore, such information in high-yielding dairy cows is needed (Sakaguchi, 2011).

Besides resumption of follicular growth, the pp period is also often characterized by cyst development (Sakaguchi et al., 2006). In early pp, 6–30% of lactating cows develop cystic follicular structures (Opsomer et al., 1996; Garverick, 1997) which can affect their subsequent fertility (Braw-Tal et al., 2009). There are many studies dealing with growth and endocrine characteristics of these structures (e.g. Hamilton et al., 1995; Hooijer et al., 2001). It is obvious that cysts are dynamic structures, as well as that follicular turnover may also happen when a cyst is present on an ovary. Also, it has been known for decades that the ovulation rate is reduced in the presence of a cyst (Aldahash and David, 1977). But to the best of our knowledge, there is no information on the growth characteristics of non-cystic follicular structures in the presence of a cyst on the ovary.

The objective of this study was to determine the indicators of follicular wave and dominant follicle developments in pp high-yielding cows during the first 60 days after parturition, and to evaluate particular follicle growth characteristics in respect to the growth wave order, the term of the first ovulation, animal parity, subsequent fertility, and also with regard to the contemporary presence or absence of a follicular cystic structure on the ovary.

MATERIAL AND METHODS

Animals

The experiment was carried out at the experimental farm of the Institute of Animal Science in Prague-Uhríněves, in accordance with the Breeding Preservation and Animal Experimentation Act No. 207/2004 Sb. The data presented in this study were collected from 24 lactating (7 primiparous and 17 multiparous) high-yielding Holstein cows with an assumed milk yield of 11 000 kg per lactation. Throughout the experiment the animals were kept in the same free stall barn, fed with the same Total Mixed Ration (TMR) balanced to their lactation status, and milked twice a day. The calving in experimental cows was assisted with gentle traction but no dystocia were recorded and there were no cases of abnormal uterine involution. The fertility of cows was evaluated on the basis of days open (DO; from parturition to successful insemination) and the frequency of hormonal treatment after the voluntary waiting period completion.

Ultrasound examinations

The reproductive organs of the cows were monitored daily from day 4–6 till day 60 pp. Ovarian and uterine structures were scanned with a real-time B-mode linear array scanner equipped with a 7.5 MHz linear rectal probe MyLab™30Vet (Esaote, Maastricht, the Netherlands). The ultrasonic images were recorded on the scanner’s hard disc, and the indicators described below were later analyzed using MyLab™Desk software developed directly for the MyLab™30Vet scanner. All ultrasonographic examinations were performed by one person after the afternoon milking. The follicular diameters presented in this paper represent the size of the antrum.
Ovarian follicular growth characteristics

Only follicles greater than 4 mm in diameter were recorded. The waves of follicular growth were retrospectively identified from the processed ultrasonographic digital video records. All follicles of at least 9 mm in diameter were defined as the dominant follicles (DFs) continuing in growth and exceeding the diameter of all other follicles in the wave. Regressive DFs (rDFs) are those which end their lifespan with regression (DF without ovulatory and cystic follicles). Ovulatory follicles (OFs) are the dominant follicles, ending their lifespan with ovulation. Assessment of DFs’ characteristics proceeded disregarding estrous cycle occurrence. Follicular wave emergence (in days pp) was determined for each individual wave and was characterized by the appearance of follicles > 4 mm. The process of DF deviation was characterized in accordance with the terms used by Ginther et al. (2003). The growth and regression periods represent the number of days during which the follicle developed from 9 mm to maximum size and subsequently diminished from maximum size to 9 mm. Growth and regression rates of DF (in mm/day) were calculated by subtracting its minimum diameter (9 mm) from its maximum one and dividing by the length of its growth or regression period (Figueiredo et al., 1997). The lifespan of DF represents the number of days which the follicle spent above the 9-mm limit. The characteristics of DFs’ development used in this manuscript is depicted in Figure 1. The emergence–deviation interval indicates the number of days from wave emergence to DF deviation. The interovulatory interval (IOI) determines the number of days between 2 consecutive ovulations. The cystic follicle was defined as an ovarian structure with a diameter of at least 18 mm developing to its maximum size in the absence of CL and being present on an ovary for at least 20 days.

Statistical analyses

In order to verify their distribution normality, the data for all analyses were examined using the Shapiro–Wilk test (Statistical Analysis System, Version 9.1.3., 2005). The data on follicular growth dynamics were analyzed by the least-square means analysis using the GLIMMIX Procedure of SAS (Statistical Analysis System, Version 9.1.3., 2005) for the main effects of parity (primiparous, multiparous) and group (G1, G2, G3), or using repeated statements for the purpose of repeated measurements respecting (Rasch and Masata, 2006). The differences among the least square means were tested at the significance level (error probability) of $P < 0.05$. Due to the fact that the levels of indicators’ “emergence” and “deviation” depend on the order of the particular follicular wave, these traits were transformed to a standard variable with normal
distribution and with the mean equal to 0 and the standard deviation equal to $1 - N(0, 1)$. The data are presented as least-square means and standard error (LSM ± SE).

**RESULTS AND DISCUSSION**

The 1st follicular wave emerged 6.6 ± 4.4 days pp in all animals. This event happened earlier in primiparous than in multiparous cows (4.7 ± 1.1 and 7.4 ± 5.0 days pp, $P = 0.09$, respectively). This trend toward earlier emergence in primiparous cows is interesting mainly with regard to a similar difference in emergence demonstrated among all the follicular waves monitored. The interval from emergence to deviation of the 1st DF was approximately 4 days (Table 1). These results are generally in accordance with the reported emergence of the 1st follicular wave pp in dairy and beef cows in days 5–10 (Savio et al., 1990a; Crowe, 2008).

Among all the 1st DFs ($n = 24$), 9 of them (37.5%) became atretic, 6 (25%) developed into a cyst, and 9 (37.5%) ovulated. The first DF ovulated in 4 out of 7 primiparous and in 5 out of 17 multiparous cows ($P = 0.22$). Ovulation of the 1st DF in 14/19, 23/50, and 6/16 Holstein cows, independently of parity, was reported by Savio et al. (1990a), Sakaguchi et al. (2004), and Kawashima et al. (2006), respectively. From the published results, it is obvious that this

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Primiparous</th>
<th>Multiparous</th>
<th>$P$-value</th>
<th>$n$</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1, emergence–deviation (days)</td>
<td>24</td>
<td>4.3 ± 0.5</td>
<td>5.1 ± 0.3</td>
<td>0.19</td>
<td>24</td>
<td>4.7 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>W2, emergence–deviation (days)</td>
<td>24</td>
<td>4.2 ± 0.2</td>
<td>4.1 ± 0.4</td>
<td>0.94</td>
<td>24</td>
<td>4.4 ± 0.3</td>
<td>4.0 ± 0.3</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>W3, emergence–deviation (days)</td>
<td>24</td>
<td>4.4 ± 0.4</td>
<td>4.2 ± 0.2</td>
<td>0.65</td>
<td>24</td>
<td>4.0 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>W4, emergence–deviation (days)</td>
<td>19</td>
<td>4.0 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>0.30</td>
<td>19</td>
<td>4.4 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>W5, emergence–deviation (days)</td>
<td>19</td>
<td>4.0 ± 0.5</td>
<td>4.3 ± 0.3</td>
<td>0.61</td>
<td>19</td>
<td>3.8 ± 0.5</td>
<td>4.4 ± 0.4</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>Days from emergence to deviation, overall</td>
<td>124</td>
<td>4.1 ± 0.2</td>
<td>4.4 ± 0.1</td>
<td>0.27</td>
<td>124</td>
<td>4.3 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>W1, No. of follicles</td>
<td>24</td>
<td>3.7 ± 0.7</td>
<td>4.4 ± 0.4</td>
<td>0.43</td>
<td>24</td>
<td>4.2 ± 0.6</td>
<td>4.0 ± 0.8</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td>W2, No. of follicles</td>
<td>24</td>
<td>3.9 ± 0.6</td>
<td>3.7 ± 0.4</td>
<td>0.84</td>
<td>24</td>
<td>3.6 ± 0.5</td>
<td>4.1 ± 0.5</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>W3, No. of follicles</td>
<td>24</td>
<td>4.3 ± 0.7</td>
<td>4.1 ± 0.4</td>
<td>0.83</td>
<td>24</td>
<td>3.3 ± 0.5</td>
<td>4.2 ± 0.2</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>W4, No. of follicles</td>
<td>18</td>
<td>4.3 ± 0.7</td>
<td>3.5 ± 0.6</td>
<td>0.42</td>
<td>18</td>
<td>3.7 ± 0.6</td>
<td>3.1 ± 0.6</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>W5, No. of follicles</td>
<td>15</td>
<td>4.5 ± 0.9</td>
<td>4.1 ± 0.6</td>
<td>0.73</td>
<td>15</td>
<td>3.8 ± 0.9</td>
<td>4.3 ± 0.7</td>
<td>5.0 ± 1.0</td>
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<tr>
<td>Number of DFs</td>
<td>127</td>
<td>5.4 ± 0.3</td>
<td>4.8 ± 0.2</td>
<td>0.22</td>
<td>127</td>
<td>5.4 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>Deviation of 1st DF (days pp)</td>
<td>17</td>
<td>8.0 ± 4.0</td>
<td>15 ± 3.0</td>
<td>0.23</td>
<td>22</td>
<td>8.0 ± 3.0</td>
<td>16 ± 5.0</td>
<td>17 ± 5.0</td>
</tr>
<tr>
<td>Deviation of 2nd DF (days pp)</td>
<td>22</td>
<td>19 ± 3.0</td>
<td>23 ± 2.0</td>
<td>0.36</td>
<td>22</td>
<td>18 ± 3.0</td>
<td>23 ± 3.0</td>
<td>27 ± 4.0</td>
</tr>
<tr>
<td>Deviation of 3rd DF (days pp)</td>
<td>23</td>
<td>27 ± 4.0</td>
<td>34 ± 3.0</td>
<td>0.19</td>
<td>18</td>
<td>28 ± 3.0</td>
<td>34 ± 3.0</td>
<td>37 ± 5.0</td>
</tr>
<tr>
<td>Deviation of 4th DF (days pp)</td>
<td>16</td>
<td>36 ± 4.0</td>
<td>40 ± 3.0</td>
<td>0.39</td>
<td>17</td>
<td>36 ± 4.0</td>
<td>40 ± 4.0</td>
<td>40 ± 7.0</td>
</tr>
<tr>
<td>Deviation of 5th DF (days pp)</td>
<td>15</td>
<td>43 ± 5.0</td>
<td>46 ± 3.0</td>
<td>0.56</td>
<td>15</td>
<td>42 ± 4.0</td>
<td>47 ± 4.0</td>
<td>41 ± 7.0</td>
</tr>
</tbody>
</table>

DF = dominant follicle, W = wave, pp = postpartum
indicator varies very widely (38–73%). Our observations are similar to those of Kawashima et al. (2006).

Based on the time of the 1st ovulation, the animals were subsequently assigned to 3 groups: group 1 (G1) – the 1st DF ovulated (n = 9), group 2 (G2) – ovulation occurred on the 2nd or later follicular waves (n = 10), and group 3 (G3) – no ovulation occurred during the experimental period (n = 5). Therefore, all the 1st ovulations came from the 1st follicular wave in G1. Representation of parities in G1, G2, and G3 was 4/5, 3/7, and 0/5 for primiparous/multiparous cows (P = 0.22). The 1st ovulation pp occurred on pp day 15 ± 4 in G1 and 38 ± 7 in G2 (P < 0.01). Cows in the G1 group did not develop any cyst, while in the G2 and G3 animals, cysts developed from the 1st DF as well as from the other DFs. In G2, the formation of cysts from the 1st DF delayed the first ovulation in 5 animals. All the animals which ovulated early pp (G1) did not even show any ovarian pathologies further in lactation. Kamimura et al. (1993a) and Kawashima et al. (2006) reported the first ovulation on day 17 ± 1 or 36 ± 4 and 17 ± 4 or 36 ± 6 pp for cows ovulating the 1st DF or the 2nd and later DFs, respectively. The difference in terms of the first ovulation between parities (primiparous 15 ± 5, multiparous 24 ± 4 days pp) was not proven to be significant (P = 0.15). Nevertheless, the trend is similar to the other indicators monitored. The mean length of IOIs (n = 11) after the 1st ovulation was 20 ± 6 days (ranging from 9 to 35 days), which is in agreement with other studies (Savio et al., 1990b; Kamimura et al., 1993b).

Based on the ultrasonographic examinations during 60 days pp, the emergence of the individual follicular waves (n = 111) occurred earlier in primiparous than in multiparous cows (a difference of 0.6 day, P = 0.0047). When the group classification was taken into account, the follicular waves (n = 111) emerged by 0.8 and 0.9 days earlier in the G1 cows than in those from the G2 or G3 groups, respectively (P = 0.0003, see Figure 2). Similarly, the process of DF (n = 93) deviation, i.e. the point when a follicle reached 9 mm in diameter, was detected by 0.5 days earlier in primiparous than in multiparous cows (P = 0.04), and DFs in the G2 and G3 cows deviated by 0.5 and 0.7 days later compared to DFs in the G1 cows (P = 0.02).

In accordance with these differences, it appears that the trend toward earlier and successful development of the 1st follicular wave in primiparous cows was subsequently projected to earlier timing of further follicular wave developments. We can see a similar trend among groups (divided according to the time of the 1st ovulation pp), when the G1 animals resumed follicular growth earlier than those in the G2 and G3 groups, and this shift is later distinct in lactation, i.e. the 1st ovulation, emergence of later waves, etc. (Table 2). Galvao et al. (2010) published a study on the positive role of the earlier 1st ovulation on subsequent fertility, which was also found in our experiment. Animals ovulating follicles from the 1st follicular wave (G1 group) got pregnant earlier (mean DO for G1, G2, and G3 were 84, 113, and 164 days, respectively; P = 0.07) and after a voluntary waiting period they were not

![Figure 2. Follicular growth wave emergence during six waves postpartum with regard to cow parity (primiparous/multiparous) and the time of the 1st ovulation (G1: the 1st dominant follicle ovulated, G2: ovulation occurred on the 2nd or later follicular waves, G3: no ovulation occurred during experimental period)
hormonally treated as often as cows ovulating later pp (frequency of hormonal treatment was 1, 8, and 5 animals for G1, G2, and G3, respectively; $P = 0.008$). Data in the literature more often describe earlier resumption of follicular growth in multiparous cows (Tanaka et al., 2008; Zhang et al., 2010). Less favourable metabolism and health status after the first delivery in primiparous cows together with synchronous progressive growth and ongoing lactation are often pointed to (Wathes et al., 2007a). Nevertheless, there are also studies where a difference dependent on parity was not found (Zain et al., 1995; Wathes et al., 2007b), as well as those which came to the opposite conclusion as we did (Kawashima et al., 2006). Therefore, this confrontation of primiparous and multiparous animals is evidently specific for individual herds. We assume that in fact the onset of follicular development is influenced rather by the ability to balance energy or stress pp under given conditions than by the parity.

Similarly, the number of follicular waves (represented by the number of developed DFs) in the entire experimental period (60 days) did not differ among parities ($5.4 \pm 0.3$ for primiparous and $4.8 \pm 0.2$ for multiparous cows), nor were they related to the time of the first ovulation pp (Table 1). Also, the interval from emergence to deviation was not influenced by the parity or time of the 1st ovulation (Table 1). Its range (approximately 4 days) is in agreement with Ginther et al. (1997). Mean numbers of follicles > 4 mm (Table 1) in individual follicular waves varied from 3.1 to 6.0, but no influence of parity or group was demonstrated. This was mainly because of high variability among cows and also among consecutive follicular waves in individual animals. Burns et al. (2005) also stated high variability among individual animals. According to them, variance in the number of follicles developed during the estrous cycle could have an important but little understood role in fertility regulation in single-ovulating species, such as cattle. Interestingly, we found the most distinct differences in the emergence-deviation interval between waves 3 and 4, where the difference in the number

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$n$</th>
<th>Primiparous</th>
<th>Multiparous</th>
<th>$P$-value</th>
<th>$n$</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st ovulation (days pp)</td>
<td>19</td>
<td>15.00 ± 5.00</td>
<td>24.00 ± 4.00</td>
<td>0.15</td>
<td>19</td>
<td>15.00 ± 4.00</td>
<td>38.00 ± 7.00</td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Maximal size of rDF (cm)</td>
<td>61</td>
<td>1.43 ± 0.06a</td>
<td>1.46 ± 0.03a</td>
<td>0.36</td>
<td>61</td>
<td>1.45 ± 0.05a</td>
<td>1.40 ± 0.04a</td>
<td>1.37 ± 0.06</td>
<td>0.68</td>
</tr>
<tr>
<td>Maximal size of OF (cm)</td>
<td>37</td>
<td>1.61 ± 0.07a</td>
<td>1.60 ± 0.06a</td>
<td>0.96</td>
<td>37</td>
<td>1.64 ± 0.06a</td>
<td>1.55 ± 0.07a</td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>Lifespan of rDF (days)*</td>
<td>55</td>
<td>13.00 ± 1.00</td>
<td>14.5 ± 0.40</td>
<td>0.39</td>
<td>55</td>
<td>14.00 ± 1.00</td>
<td>15.00 ± 1.00</td>
<td>14.00 ± 2.00</td>
<td>0.80</td>
</tr>
<tr>
<td>Growth period of rDF (days)**</td>
<td>60</td>
<td>6.60 ± 0.90</td>
<td>7.30 ± 0.50</td>
<td>0.52</td>
<td>60</td>
<td>7.40 ± 0.80</td>
<td>7.00 ± 0.70</td>
<td>7.10 ± 0.90</td>
<td>0.94</td>
</tr>
<tr>
<td>Growth period of OF (days)**</td>
<td>37</td>
<td>6.50 ± 0.60</td>
<td>6.60 ± 0.50</td>
<td>0.94</td>
<td>37</td>
<td>6.90 ± 0.60</td>
<td>6.20 ± 0.70</td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>Regression period of rDF (days)</td>
<td>55</td>
<td>5.80 ± 0.80</td>
<td>6.60 ± 0.50</td>
<td>0.37</td>
<td>55</td>
<td>5.70 ± 0.70</td>
<td>6.60 ± 0.60</td>
<td>6.80 ± 0.80</td>
<td>0.37</td>
</tr>
<tr>
<td>Growth rate of rDF (cm/day)</td>
<td>60</td>
<td>0.07 ± 0.02b</td>
<td>0.09 ± 0.01b</td>
<td>0.27</td>
<td>60</td>
<td>0.09 ± 0.01b</td>
<td>0.09 ± 0.01b</td>
<td>0.08 ± 0.02</td>
<td>0.85</td>
</tr>
<tr>
<td>Growth rate of OF (cm/day)</td>
<td>37</td>
<td>0.12 ± 0.01b</td>
<td>0.13 ± 0.01b</td>
<td>0.86</td>
<td>37</td>
<td>0.12 ± 0.01b</td>
<td>0.13 ± 0.01b</td>
<td></td>
<td>0.57</td>
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<tr>
<td>Regression rate of rDF (cm/day)</td>
<td>55</td>
<td>0.07 ± 0.20</td>
<td>0.09 ± 0.10</td>
<td>0.64</td>
<td>55</td>
<td>0.08 ± 0.20</td>
<td>0.07 ± 0.10</td>
<td>0.10 ± 0.20</td>
<td>0.67</td>
</tr>
</tbody>
</table>

rDF = regressive dominant follicle, OF = ovulatory dominant follicle, pp = postpartum

*interval when the rDF size was over the 0.9 cm limit

**from deviation (0.9 cm) to maximal size (rDF) or ovulation (OF)

a,bdata in the column signed with the same superscripts differ significantly at $P < 0.05$
of follicles also approached the level of statistical significance.

Nevertheless, it is obvious that taking into account all the above-mentioned data, the rhythm of follicular wave development was generally similar in all the animals. Therefore, in accordance with the opinion of Velazquez et al. (2008), it is obvious that prolongation of the non-ovulatory period pp is not a result of DF absence.

The indicators of follicular development after DF deviation – the mean maximum diameters of regressive DFs (rDFs) or ovulatory follicles (OFs) and the lengths of rDF or OF growth periods are presented in Table 2. No differences were found among parities or the G1, G2, and G3 groups (the 1st ovulation until day 20 pp, in the range of 20–60 days, or later than in 60 days, respectively). Nevertheless, the rDFs were smaller than the OFs (approximately 1.4 vs. 1.6 cm, \( P < 0.01 \)), similar to the data presented by Savio et al. (1990b) or Sartori et al. (2004), and the growth rate was faster in OFs than in rDFs (\( P < 0.05 \)). The differences in maximum size and growth rate between rDFs and OFs (both \( P < 0.05 \)) were also evident in the milieu with a follicular cyst (Table 3). Interestingly, the length of the growth period did not reflect the size and growth rate differences – rDFs 7.1 ± 0.8, OFs 6.5 ± 0.6 days (\( P = 0.43 \)). These values are in accordance with the data of Siros and Fortune (1988), Savio et al. (1988), and Ginther et al. (1989), but all these results were obtained in heifers not influenced by lactation and, on the contrary, the data on lactating cows by Savio et al. (1990b) indicated rather by 2 days shorter growth periods. Nevertheless, the reason for such a difference is more probably in the methodology of ultrasound examination, as Savio et al. (1990b) reported follicular wave emergence on days 2–6 after ovulation. This is not in agreement with repeatedly observed first wave emergence on the 1st–2nd days after ovulation (Ginther et al., 1989; Šichtař et al., 2010). Also, Savio et al. (1990b) found the lifespan of rDFs to be evidently shorter than we did (Table 2), although they monitored the follicles until their disappearance, whereas we stopped their monitoring when their size dropped back to 9 mm. Therefore, we assume in fact the pattern of rDF/OF growth is probably similar in cows and heifers. On the other hand, we did not mention any larger variability in OF growth addressing the differences regularly observed in 2-wave or 3-wave cycles (generally 9–10 days vs. 5–7 days; Šichtař et al., 2010). We believe that this is due to the fact that most OF monitored in our experiment grew without the influence of CL, therefore independently of wave patterns or estrous cycle stages. This points out the role of CL in follicular growth modulation, in its shortening and lengthening, as mentioned by Wilson et al. (1998).

Similarly to previous indicators of rDF/OF growth, their growth rates were not influenced by parity or group (Table 2). Our values, especially for rDFs, are lower compared to those of many other studies. Interestingly, except for an article by Gaur and Purohit (2007), these papers were published about

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OF (n = 37)</th>
<th>P-value</th>
<th>rDF (n = 71)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predisposition of ovulation occurrence in a group*</td>
<td>2.00 ± 0.10</td>
<td>1.20 ± 0.10</td>
<td>&lt; 0.01</td>
<td>2.27 ± 0.16</td>
</tr>
<tr>
<td>Deviation (days)**</td>
<td>0.49 ± 0.29</td>
<td>–0.18 ± 0.17</td>
<td>0.06</td>
<td>0.62 ± 0.22</td>
</tr>
<tr>
<td>Maximum size (cm)</td>
<td>1.60 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.60 ± 0.09&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.95</td>
<td>1.40 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Growth (days)</td>
<td>6.80 ± 0.60</td>
<td>6.00 ± 0.85</td>
<td>0.44</td>
<td>7.43 ± 0.54</td>
</tr>
<tr>
<td>Growth rate (cm/day)</td>
<td>0.15 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11 ± 0.01&lt;sup&gt;e,g&lt;/sup&gt;</td>
<td>0.04</td>
<td>0.08 ± 0.01&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Regression (days)</td>
<td>54 6.20 ± 0.50</td>
<td>6.20 ± 0.90</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Regression (cm/day)</td>
<td>54 0.09 ± 0.01</td>
<td>0.05 ± 0.03</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>

*OFs were evaluated in groups 1 and 2 only
**standard variables with normal distribution, where given values represent deviation from 0
<sup>a–d</sup>data in the row signed with the same superscript differ significantly at \( P < 0.05 \)
20 years ago (e.g. Savio et al., 1988) and usually demonstrated surprisingly late emergence especially of the 1st follicular growth waves and a short period of follicular growth. More recent papers deal with data much more similar to ours, but unfortunately they focus on OFs only (e.g. Carvalho et al., 2008; Morris et al., 2009). The growth rate of OFs was significantly higher than that of rDFs in our animals ($P < 0.05$), consistently among primiparous as well as multiparous, G1 or G2 animals (Table 2). This is in accordance with data by Murphy et al. (1990) and Adams et al. (2008). Nevertheless, many other studies did not demonstrate such a difference (Savio et al., 1990b) or found an opposite relationship (Savio et al., 1988; Ginther et al., 1989). Whether the different methodological approach mentioned above, genetic selection progress made in the past 20 years, together with negative trends in reproductive efficiency and ovarian activity (e.g. Garmo et al., 2009; Kafi and Mirzaei, 2010), or simply physiological variation is responsible for these result variabilities – all this is questionable.

Cysts

Among all the animals ($n = 24$), 42% of them ($n = 10$) developed a cyst during the monitored period. Moreover, two of the cows developed an additional cyst. Such incidence corresponds to the data of similarly based studies (44%, Hamilton et al., 1995; 27%, Vanholder et al., 2005; 71%, Sakaguchi et al., 2006). Also, the repeated presence of cysts is a well-known phenomenon (Hamilton et al., 1995; Kengaku et al., 2007). In six cases, the cyst developed as early as the 1st follicular wave and persisted until the monitoring ended (day 60 pp). Although such occurrence of cysts developing from the 1st detected DF is not surprising, as Savio et al. (1990a) found the same ratio (21%), their long persistence is interesting namely in terms of routine ovary function examination at the end of the voluntary waiting period.

All together 108 follicles (rDFs + OFs) were monitored. 15 out of 71 rDFs and 12 out of 37 OFs developed when a cyst was present on the ovary. Such a rate is significantly ($P < 0.0001$) lower in both follicle types than one would expect on the basis of time periods when the follicles could develop in the presence or absence of a cyst (Table 3). Significantly fewer rDFs or OFs developed when a growing cyst was present than in the absence of a cyst ($P < 0.0001$), but also, than in the presence of a stagnating cyst ($P < 0.0006$). If a stagnating cyst was present on an ovary, the incidence of OFs was comparable to the situation where cysts were absent; however, non-ovulating rDFs appeared less frequently ($P < 0.004$). The shift in the rDF/OF ratio in such a milieu is also highly significant ($P < 0.0001$).

Follicle development in the presence of a cyst was presumed on the basis of CL presence in a study by Aldahash and David (1977). It is interesting that many articles discuss the characteristics of cyst development (e.g. Sakaguchi et al., 2006), but the development of follicles under cystic conditions is not mentioned. The graphic schemes in Sakaguchi et al. (2006) clearly indicate that follicles are able to grow in the presence of cysts. Unfortunately, the authors did not monitor the follicular growth/developmental characteristics. Hence, there is still a lack of information in this area (Sakaguchi, 2011). According to our data, it seems that follicular growth restraint in the presence of a growing cyst was blocked out almost immediately after cessation of the cyst growth. This could be a consequence of steroidogenic cyst activity typical for the period of the cyst growth (Noble et al., 2000; Silvia et al., 2002). Moreover, follicular development after cyst growth cessation seems to be somewhat stimulated, as the following follicular growth wave generated the OF in all cases (7 animals) except for 3 animals which were not able to ovulate during the entire 60-day monitored period. Even though Sakaguchi et al. (2006) did not emphasize this result, it is obvious from their graphs that they found a similar pattern in 4 out of 5 animals, as well.

This hypothesis of an accelerated follicular development is also supported by analysis of follicular growth characteristics in relation to the presence or absence of a cyst (Table 3). Although the rDFs were not influenced by these aspects, the OFs' growth rates (cm/day) were faster in the presence of a cyst ($P = 0.04$).
Although Table 3 indicates some tendency to a later deviation of OFs in the presence of a cyst, analysis of the effect on the individual animals revealed that the deviation is in fact dependent on the differences in follicular wave timing between animals with or without any cyst occurrence during the entire pp period (with cysts 0.6 day later, \( P = 0.005 \)).

**CONCLUSION**

Based on the indicators of the 1st follicular wave development such as its emergence, fate of the 1st DF, time of the 1st ovulation, or the 1st IOI length, the animals in our experiment reached average reproductive efficiency.

The rhythm of follicular waves was quite uniform up to DF deviation, regardless of whether or not they reached ovulation (except in cows with cysts). Small differences were notable in their timing only (earlier in primipara or G1, later in cows developing a cyst). Therefore, prolongation of the non-ovulatory period pp in individual animals was not a result of DF absence.

Although some indicators were found to be significantly better in primipara, in the light of published data we assume that this effect is specific rather for the herd and the commencement of follicular development is influenced rather by the ability to balance energy or stress pp under given conditions than by the parity.

The earlier 1st ovulation was connected with earlier timing of follicular wave growth but, especially, the final cow reproductive performance was related to the fate of the 1st DF – animals ovulating early (i.e., within 20 days) pp showed significantly better fertility than those ovulating later in lactation (earlier pregnancy and less hormonal treatment).

After dominant follicle deviation, the indicators of follicular development (e.g., growth rate) differed in depending on follicle type (rDF vs. OF). Therefore, in interpreting results from other studies, it is necessary to distinguish which follicle type they are focusing on.

Development of follicles was dramatically suppressed beyond, but not before, deviation in the milieu of a growing cyst. Meanwhile, cessation of cyst growth accelerated the development of OF. On the contrary, a cystic structure without any significant growth can persist for weeks with no effect on follicular development.

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