A comparison of the follicular dynamics in heifers of the Czech Fleckvieh and Holstein breeds

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ABSTRACT: Differences in follicular development and repeatability of follicular growth pattern among Czech Fleckvieh (*n* = 20) and Holstein (*n* = 23) heifers were investigated. Follicular dynamics was evaluated by daily sonographic scanning during three interovulatory intervals. The mean duration of the interovulatory interval was 20.66 ± 0.32 days, no differences between breeds were observed. The proportion of the non-alterna-ting pattern was nearly the same as that of the alternating pattern (54% and 46%, respectively). The majority of IOIs ≤ 21 days were of the 2-wave pattern (71%) whereas only 29% of them were of the 3-wave pattern. Conversely, the majority of IOIs ≥ 22 days were of the 3-wave pattern (84%), whereas only 16% were of the 2-wave pattern. Differences could be observed in the Czech Fleckvieh heifers. Comparing 2- and 3-wave interovulatory intervals, 44.2% of the heifers exhibited 3 waves and 55.8% of the heifers exhibited 2 waves of follicular growth. The ratio of 3- to 2-wave heifers was about the same in the Holstein breed; in Czech Fleckvieh 2-wave cycles slightly dominated (11/12, 8/12; respectively). In Holstein heifers, the first follicular wave occurred 0.92 ± 0.15 days after ovulation in 2-wave interovulatory intervals, and the emergence of the first wave in 2-wave Czech Fleckvieh heifers appeared later ($P < 0.05$), 1.83 ± 0.3 days after ovulation. The maximal size reached by the dominant follicles in all animals and in the Czech Fleckvieh differed in the first and in the second wave of 2-wave cycles ($P < 0.05$). In 3-wave interovulatory intervals the dominant follicles in the second wave differed ($P < 0.05$) from the mean diameters of the first and the third wave in the Czech Fleckvieh. The ovulatory follicles were significantly ($P < 0.05$) smaller in 2-wave than in 3-wave interovulatory intervals among all animals and between the Holstein and Czech Fleckvieh heifers. In conclusion, we found a similar pattern of ovarian follicular dynamics in Czech Fleckvieh and Holstein heifers kept under identical nutritional and environmental conditions. Whether the significant difference in the emergence of the 1st follicular wave in 2-wave IOIs between C and H heifers is of real biological significance is ambiguous.

Keywords: cattle; oestrous cycle; bovine ovary; ovarian follicular wave; repeatability

The fertility of cows has become a major problem in the last 20 years, as reported by recent studies (e.g. Dobson et al., 2007). An elementary part of reproduction management in a herd is also precise reproduction in heifers, and some reproductive disorders can be seen even in heifers.

There are many factors affecting the reproductive efficiency of heifers, for example body weight (Chebel et al., 2007), metabolic status (Bergfeld et al., 1994; Ferguson, 2005), BCS (Sejrsen et al., 1999), heat stress (Wilson et al., 1998a) and sufficient ovarian activity (Fortune, 1993).

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Ovarian activity can be evaluated in terms of the following indicators: length of interovulatory interval (IOI), wave pattern (mostly 2 vs. 3 waves of follicular growth per IOI), time of emergence of follicular wave, number of follicles in each wave, mean diameter, and growth rate of dominant and ovulatory follicles (Sartori et al., 2004).

It has been determined that ovarian follicles in peripubertal heifers grow and regress in a wave-like manner (Bleach et al., 2004) and that the majority of bovine oestrous cycles (i.e. > 95%) are composed of either two or three follicular waves (Adams et al., 2008). A wave of follicular growth involves the synchronous development of a group of follicles (Driancourt, 2001) and is characterized by the recruitment, selection and dominance of the follicle (Hooper et al., 1993; Driancourt, 2001).

It is well established that Holstein heifers mostly exhibit two or three waves of follicular development in each IOI (Savio et al., 1988; Ginther et al., 1989). Nevertheless, some papers describe the incidence of 1- or 4-wave IOIs (Savio et al., 1988; Sirois and Fortune, 1988). Fortune (1993) and Wolfenson et al. (2004) evaluated 3-wave IOIs as the more frequent type of follicular growth in Holstein heifers. On the other hand, Kulick et al. (2001) and Sartori et al. (2004) recorded a higher percentage of Holstein heifers with 2-wave IOIs. In beef cattle Ahmad et al. (1997) reported 2-wave IOIs with a higher incidence (86%). The factors that determine whether an animal will have two or three waves of follicle development are not understood. There even arises a question whether the number of follicular waves is constant within individual animals. Price and Carriere (2004) demonstrated that Holstein heifers are not always "two-wave" or "three-wave" individuals and may switch between cycles with two and three waves of follicular growth per interovulatory interval.

All the above-mentioned facts are well described in Holstein heifers and cows (e.g. Sirois and Fortune, 1988; Sartori et al., 2002; Wolfenson et al., 2004). Significantly fewer experiments have been performed on beef heifers (Hooper et al., 1993; Bergfeld et al., 1994; Evans et al., 1994; Gasser et al., 2006) and we found no paper dealing with dual-purpose breeds of cattle. Czech Fleckvieh heifers rank among dual-purpose breeds, reaching puberty at 8–10 months, weighing 340–360 kg at 12 months, and with milk yield of 5 800 kg at the first lactation.

Reproductive efficiency is slightly different between Holstein and Czech Fleckvieh heifers in the Czech Republic (e.g. conception rate after first AI). This could be due to the number of follicular waves per IOI (Townson et al., 2002; Celik et al., 2005), but the results are inconsistent. In lactating dairy cattle, IOIs that consist of at least three waves of follicular growth preceding ovulation and insemination appear to be conducive to improved fertility (Townson et al., 2002; Celik et al., 2005).

According to results presented in Ahmad et al. (1997) and Jaiswall et al. (2009) we hypothesize that dual-purpose heifers should exhibit 2-wave patterns more often than 3-wave patterns. The primary objective of this study was to compare various indicators: length of interovulatory interval, wave pattern (i.e. 2- vs. 3-wave), time of emergence of follicular wave, number of follicles in each wave, mean diameter of dominant (DFs) and ovulatory follicles in 2- or 3-wave heifers kept under the same nutritional and environmental conditions and to determine if these parameters are influenced by the breed (Holstein and Czech Fleckvieh). The secondary objective was to determine if 2- or 3-wave IOIs are repeated under the same herd conditions.

MATERIAL AND METHODS

Animals

The experiment proceeded in accordance with Decree No. 207/2004 on the Protection, Breeding and Utilization of Experimental Animals. For the experiment 51 heifers from an experimental herd were selected while eight heifers were discarded during the trial, six heifers due to the narrow rectum and two animals that did not show any cyclic activity. Finally in the group of all experimental animals (n = 43), Holstein (n = 23; group H) and Czech Fleckvieh heifers (n = 20; group C) at the mean age of 12 months (10–13 months) and with at least one detected peak of walking activity were monitored. During the period of heat, the animals were observed for signs of oestrus 3 times daily. As a control, peaks of walking activity from the AfiFarm^{TM} (S.A.E. AFIKIM, ISR) system were monitored. All the heifers were weighed at the beginning, in the middle and at the end of the experiment. Their average weight at the beginning of the experiment was 360 kg (292–437 kg), with an average increase in weight of 24.5 kg per month. The animals of both breeds were housed together. The stable was equipped with free straw-bedded stalls and outdoor run. The heifers were fed under standard conditions – TMR feeding twice daily with free access to water. In the Czech Fleckvieh (*n* = 6) and in the Holstein (*n* = 7) heifers the repeatability of the follicular growth wave pattern was examined during two consecutive interovulatory intervals.

Sonographic examinations

Ovarian follicles were monitored with a real-time B-mode linear array ultrasound scanner equipped with a 7.5 MHz linear rectal probe (MyLab™30Vet, Esaote, NL). Sonographic examinations were performed daily from the beginning of the cycle (Day 0: day of ovulation) under optimal conditions in accordance with the method of Quirk et al. (1986). Only in the third IOI was repeatability of the pattern of follicular growth examined by scanning performed every other day. There was no drug treatment before or during the experiment. Ultrasonic images of each ovary were recorded on the hard disk of MyLab30Vet. Some parameters described below were evaluated on a PC with MyLab™Desk software developed directly for the MyLab™30Vet ultrasound scanner. The waves of follicular growth were identified retrospectively from the processed sonographic digital video records. All sonographic examinations were processed by one person, and the follicular diameters described in this paper represent the size of the antrum.

Reproductive management

The length of IOI was determined as the interval between 2 consecutive ovulations. A heifer was said to be in oestrus (day of oestrus = D 0 of the oestrous cycle) when she remained immobile while mounted by another female. The dominant follicle (DF) of a wave was defined as the one that measured at least 0.9 cm in diameter and exceeded the diameter of all other follicles in the wave. The follicular wave was characterized by the emergence of follicles ≥ 4 mm, and the wave was said to be at an end when the DF ovulated or became atretic. The interovulatory intervals were classified into the following groups: A/ 2-wave interovulatory interval (the first wave with a dominant anovulatory follicle and the second wave with an ovulatory follicle), B/ 3-wave interovulatory interval (the first and the second wave with a dominant anovulatory follicle and the third wave with a dominant ovulatory follicle).

Table 1. Results (mean ± SEM) comparing all heifers (*n* = 43) with typical cycles (2-wave or 3-wave) for follicular development

Parameters	IOIs	
	2-wave cycles	3-wave cycles
Number of animals	24	19
Interovulatory interval (days)	20.0 ± 0.29[a]	21.84 ± 0.52[a]
Emergence of wave 1 (day of cycle)*	1.46 ± 0.20	1.79 ± 0.21
Emergence of wave 2 (day of cycle)*	10.67 ± 0.19[b]	9.37 ± 0.32[b]
Emergence of wave 3 (day of cycle)*		16.95 ± 0.53
No. of follicles e” 4 mm	2.87 ± 0.38	2.8 ± 0.39
Maximal size of DFs in wave 1 (mm)	1.31 ± 0.03[1]	1.24 ± 0.03[1]
Maximal size of DFs in wave 2 (mm)	1.4 ± 0.02[1]	1.08 ± 0.02[2]
Maximal size of DFs in wave 3 (mm)		1.21 ± 0.04[1]
Maximal size of ovulatory follicles (mm)	1.37 ± 0.03[c]	1.15 ± 0.04[c]

*Day 0 = ovulation

[a,b,c]data with the same superscripts within the same row differ significantly ($P < 0.05$)

[1,2]data with different superscripts within the same column differ significantly ($P < 0.05$)

Statistical analysis

The study was performed in three replications. We did not find any differences among the replications so we evaluated these data together. All data were analyzed using the Statistica Software ver. 8 (Statsoft, CZ). Follicular data were timed to the day of ovulation (Day 0). When 2 groups of variables were compared, Student's *t*-test was used for data evaluation. Before Student's *t*-test was used, normality and homogeneity were tested. Once normality was affected, the Mann-Whitney test was used instead. In the case of 3 groups of variables, Cochran's test and Shapiro-Wilk test were used for normality and homogeneity evaluation. The one-way ANOVA with subsequent Fisher's test for multiple comparisons was used. The proportion of IOIs ≤ 21 days and IOIs ≥ 22 days was compared using Chi-square analysis. When the number of follicles was analyzed statistically, we used only 30 heifers, because in the third IOI monitored in this experiment we scanned the ovaries every other day. All the conclusions were established on a $P < 0.05$ level of significance and all data are presented as means ± SEM.

RESULTS

The mean duration of IOI in the group of all experimental animals (n = 43) was 20.66 ± 0.32 days. The duration of IOIs between Holstein (n = 23; H) and Czech Fleckvieh (n = 20; C) heifers did not differ. Among all the animals, heifers exhibiting 2 waves of follicular growth showed the IOIs 1.84 days shorter ($P < 0.05$) than heifers with 3-wave IOIs (Table 1). In H heifers the length of 2-wave IOIs was 2.08 days shorter ($P < 0.05$) than that of 3-wave IOIs. We found a similar trend in C heifers (Table 2).

As shown in Table 3, the majority of IOIs ≤ 21 days were of the 2-wave pattern, 71%, whereas only 29% of them were of the 3-wave pattern. Conversely, the majority of IOIs ≥ 22 days were of the 3-wave pattern (84%), whereas only 16% of them were of the 2-wave pattern. It is obvious that the ratios at the level of all experimental animals are nearly the same as at the level of the individual breeds (H and C).

Seven of the 13 heifers (54%) examined through 2 consecutive IOIs for repeatability of the pattern of follicular growth exhibited a consistent wave pattern of follicular growth (Figure 1). Five heifers (39%) exhibited a consistent 2-wave pattern and two (15%) heifers exhibited a consistent 3-wave pattern. Six of the 13 heifers (46%) alternated between the 2- and 3-wave pattern during two consecutive IOIs. Of the animals that alternated, two displayed a 2-wave IOI followed by a 3-wave IOI, and four displayed a 3-wave IOI followed by a 2-wave IOI. The proportion of non-alternating patterns was nearly

Table 2. Follicular characteristics of 2- versus 3-wave interovulatory intervals (IOIs, mean ± SEM) in group C (n = 20) and H (n = 23) of experimental animals

Parameters	2-wave cycles		3-wave cycles	
	C (n = 12)	H (n = 12)	C (n = 8)	H (n = 11)
Interovulatory interval (days)	20.0 ± 0.39^{a}	19.83 ± 0.3^{b}	21.75 ±0.62^{a}	21.91 ± 0.80^{b}
Emergence of wave 1 (day of cycle)*	1.83 ± 0.30^{a}	0.92 ± 0.15^{a}	1.5 ± 0.27	2.0 ± 0.30
Emergence of wave 2 (day of cycle)*	10.92 ± 0.20^{a}	10.33 ± 0.26	8.8 ± 0.37^{a}	9.82 ± 0.44
Emergence of wave 3 (day of cycle)*			16.88 ± 0.77	17.0 ± 0.75
No. of follicles e” 4 mm	2.78 ± 0.52	3.0 ± 0.58	3.0 ± 0.55	3.2 ± 0.25
Maximal size of DFs in wave 1 (mm)	1.29 ± 0.04^{1}	1.33 ± 0.04	1.26 ± 0.05^{1}	1.23 ± 0.05
Maximal size of DFs in wave 2 (mm)	1.41 ± 0.03^{2}	1.4 ± 0.04	1.1 ± 0.04^{2}	1.07 ± 0.03
Maximal size of DFs in wave 3 (mm)			1.24 ± 0.05^{1}	1.19 ± 0.06
Maximal size of ovulatory follicles (mm)	1.35 ± 0.05^{a}	1.37 ± 0.04^{b}	1.15 ± 0.04^{a}	1.15 ± 0.07^{b}

*Day 0 = ovulation

^{a,b}data with the same superscripts within the same row differ significantly ($P < 0.05$)

^{1,2}data with different superscripts within the same column differ significantly ($P < 0.05$)

Table 3. The percentage representation of 2-wave and 3-wave IOIs in intervals ≤ 21 or ≥ 22 days in the group of all experimental animals (n = 43), C (n = 20), and H (n = 23) group of animals

IOI	Group of heifers					
	all experimental animals		C		H	
	2-wave IOI	3-wave IOI	2-wave IOI	3-wave IOI	2-wave IOI	3-wave IOI
≤ 21 days	71% (22/31)[a]	29% (9/31)[b]	78.6% (11/14)[c]	21.4% (3/14)[d]	64.7% (11/17)	35.3% (6/17)
≥ 22 days	16% (2/12)[a]	84% (10/12)[b]	16.7% (1/6)[c]	83.3% (5/6)[d]	16.7% (1/6)	83.3% (5/6)

[a,b,c,d]data with the same superscript in the same column differ significantly ($P < 0.05$)

the same as that of alternating patterns (7/13, 54%; and 6/13, 46%, respectively).

During the whole experiment we observed 4 different types of follicular development based on the number of follicular waves per IOI: 1 wave (n = 1), 2 waves (n = 24), 3 waves (n = 19) or 4 waves (n = 1). Comparing 2- and 3-wave IOIs, 44.2% of the heifers exhibited 3-wave and 55.8% exhibited 2-wave patterns of follicular development (Table 1). The number of follicular waves per one IOI differed between the H and C animals. The proportion of 3- and 2-wave IOIs in H heifers was about the same, whereas in C heifers 2-wave cycles (Table 2) slightly dominated.

The emergence of the 1st and 2nd wave of follicular development per IOIs in all animals is described in Table 1. The emergence of the first, second and third wave of follicular growth in C and H heifers is shown in Table 2. In H heifers, the first wave occurred 0.92 ± 0.15 days after ovulation in 2-wave IOIs, and the emergence of the first wave in the 2-wave C heifers appeared later ($P < 0.05$), 1.83 ± 0.3 days after ovulation.

We found no differences in the number of follicles among the follicular growth waves in all experimental animals (Table 1) and in C and H (Table 2).

The maximal size reached by dominant follicles (DFs) differed in all animals (Table 1) between the first and the second wave of 2-wave IOIs ($P < 0.05$). In 3-wave IOIs the DFs of the second wave of follicular growth were significantly ($P < 0.05$) smaller than those in the two other waves. In H heifers exhibiting 2-wave IOIs, the first and the second DFs reached very similar diameters (Table 2). The maximal size of the DFs of groups C and H is shown in Table 2. In C heifers with 2-wave IOIs, the sizes of the DFs in the first and the second wave differed significantly ($P < 0.05$). In 3-wave IOIs the DFs in the second wave differed ($P < 0.05$) from the mean diameters of the first and the third wave in C heifers.

The ovulatory follicle in all experimental animals was significantly ($P < 0.05$) larger in 2-wave IOIs than in 3-wave IOIs (Table 1). If we focus on differences between breeds, the data on both breeds show similar trends like all experimental animals

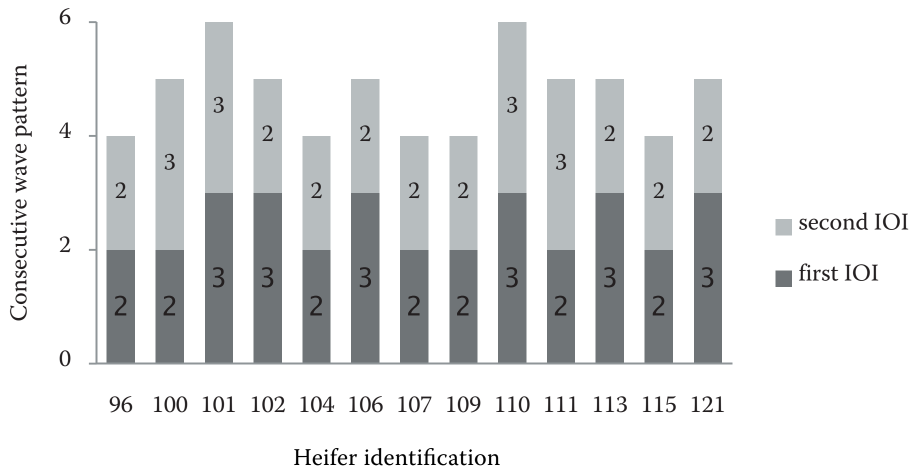

Figure 1. Distribution of 2- and 3-wave interovulatory intervals (IOIs) in heifers (n = 13) examined during consecutive IOIs

and exhibit significant ($P < 0.05$) differences in the diameter of the ovulatory follicle between 2-wave and 3-wave IOIs.

DISCUSSION

To our knowledge, this is the first complete description and comparison of ovarian follicular dynamics during the bovine oestrous cycle based on sonographic analyses, between dairy (Holstein) and dual-purpose (Czech Fleckvieh) breeds.

Current evidence (Cooperative Regional Project, 1996; Gasser et al., 2006; Adams et al., 2008) indicates that ovarian follicles grow and regress in a wave-like manner. It is obvious that the length of IOI highly corresponds to the number of waves per IOI (Jaiswal, 2007; Jaiswal et al., 2009). In our experiment the mean length of IOI for all cycles monitored was 20.5 days, which is identical with some other reports (Sirois and Fortune, 1988; Price and Carriere, 2004; Sartori et al., 2004). Wolfeson et al. (2004) reported 22 ± 0.4 days in coeval Holstein heifers, but these data are quiet scarce. It is well established that heifers with 3 follicular waves tend to have a longer IOI than females of the same category with 2 waves as a consequence of the delayed time of luteolysis (Sartori et al., 2004). Differences as large as 3.3 and 2.4 days were described by Price and Carriere (2004) and Sartori et al. (2004), respectively. The data in the present study conform to these findings; the differences among heifers in the group of all experimental animals were 1.84 days and between the H and C group they were 2.08 and 1.75 days, respectively.

In our study we demonstrated that the wave pattern (2- or 3-wave pattern) in two consecutive IOIs is repeatable in 54% (n = 7) and random in 46% (n = 6) of heifers. This finding is in contrast to Jaiswall's (2007) conclusion when the occurrence of 2- and 3-wave patterns was reported as highly repeatable (70% of all IOIs). Nevertheless, Price and Carriee (2004) reported a similar percentage (55%) of random wave patterns to ours. This 50% variability can cause the equivocation of representation of 2- or 3-wave patterns in IOIs by some authors who describe 2-wave IOIs (Knopf et al., 1989; Ko et al., 1991; Rajamahendran and Taylor, 1991; Ginther et al., 1996; Wilson et al., 1998a; Roth et al., 2000; Kulick et al., 2001; Townson et al., 2002) as the more frequent pattern of follicular growth than 3-wave IOIs (Ireland, 1987; Fortune et al., 1988; Savio et al., 1988; Sirois and Fortune, 1988).

The number of waves of follicular growth during the IOI is variable among heifers. Some authors report a predominance of the 2-wave pattern of follicular growth (Ginther et al., 1996; Wilson et al., 1998a; Roth et al., 2000; Kulick et al., 2001;) while others report a preponderance of the 3-wave pattern (Ireland, 1987; Fortune et al., 1988; Savio et al., 1988; Sirois and Fortune, 1988). In this study we even observed one IOI with only one wave of follicular growth and another IOI with four waves. These IOIs terminated in the ovulation of dominant follicle. Sirois and Fortune (1988) and Bleach et al. (2004) found heifers exhibiting four waves of follicular growth, whereas Evans et al. (1994) mentioned heifers exhibiting only one wave of follicular growth per IOI. Nevertheless, it is possible to say that the overwhelming majority of IOIs consist of either 2 or 3 waves of follicular growth. H heifers in this study exhibited 2 and 3 waves at an equal ratio (11:12), whereas under the same conditions in C heifers the IOIs consisted mainly of those with 2 waves of follicular growth (the ratio of 2- to 3-wave IOIs was 12:8). Interestingly, Evans et al. (1994) published the same ratio in Hereford heifers. Similarly, Ahmad et al. (1997) and Jaiswall et al. (2009) acknowledged more frequent occurrence of 2-wave IOIs in beef (86%) and cross Hereford heifers (68%), respectively.

According to Zeitoun et al. (1996) and Driancourt (2001), the number of waves is not influenced by the age or the year season. However, Wilson et al. (1998b) reported that the proportion of 3-wave IOIs increased in heifers subjected to heat stress. Our laboratory performed this experiment in September, May and June, when the average temperatures were 16.7°C, 14.4°C and 17.7°C, respectively. Therefore, the influence of a hot summer season is less probable. Other known factors influencing the number of growth waves per IOI such as nutrition (Murphy et al., 1991; Chelikani et al., 2003), parity and the character of lactation (Lucy et al., 1992) could not influence the differences shown in this study.

There is a more or less uniform opinion in the literature on the emergence of follicular waves during IOI. Generally, follicles in the 1st wave develop in 2-wave IOIs for 10 days, while in 3-wave IOIs it is 7 days, and the emergence of the 2nd wave begins earlier in 3-wave IOIs and later in 2-wave IOIs (Sartori et al., 2004). The emergence of the 1st wave in 2-wave IOIs occurs on the 2nd day, and the ovulatory wave emerges on the 10th day (Sirois and Fortune, 1988; Ginther et al., 1989; Enright et al., 2002). In 3-wave IOIs the particular follicular growth waves emerge on the 2^{nd}, 9^{th} and 16^{th} day of IOI (Sirois and Fortune, 1988; Ginther et al., 1989; Sartori et al., 2004; Wolfenson et al., 2004). Our results support this concept. The waves of follicular growth in 2-wave IOIs in the group of all experimental animals emerged on the 1.46^{th} and 10.67^{th} day and in 3-wave IOIs the first, second and third wave emerged on the 1.79^{th}, 9.37^{th}, and 16.95^{th} day, respectively.

Nevertheless, Sartori et al. (2004) found statistically conclusive differences in the emergence of the 2^{nd} wave between 2-wave and 3-wave IOIs in Holstein heifers. This relationship was also evaluated as statistically significant in our experiment. Moreover, we found a significant difference on the breed level in the emergence of the 1^{st} wave of 2-wave IOIs, when the 1^{st} wave emerged earlier in H heifers than in C heifers (day 0.92 vs. day 1.83, respectively). Interestingly, intervals between the emergence of the 1^{st} and 2^{nd} wave in 2-wave IOIs are nearly the same (9.41 vs. 9.09 days, H vs. C respectively).

The numbers of follicles (≥ 4 mm) in this experiment, recorded in each individual growth wave, seemed to be smaller when compared to data mentioned by Sirois and Fortune (1988) or Hooper et al. (1993). Ginther et al. (1996) reported even eleven follicles (≥ 4 mm) in one growth wave. It is known that the number of growing follicles influences the size of the ovaries. Nevertheless, our data are consistent among breeds as well as among experiments, and we found great numbers of follicles < 4 mm on the ovaries of all heifers.

Dominant follicles reached different proportions in the final phase of growth in this study when 2-wave and 3-wave animals were compared. In 2-wave IOIs dominant follicles grew to a larger size than in 3-wave IOIs, which is similar to results published in literature (Sirois and Fortune, 1988; Townson et al., 2002; Wolfenson et al., 2004). We can find this phenomenon described not only in heifers but even in cows (Townson et al., 2002; Celik et al., 2005). Interestingly, in this experiment the difference was significant in C heifers; in H heifers we could observe only an insignificant trend.

Concerning the size of dominant follicles in 2-wave cycles, we measured smaller diameters in wave one than in wave two in heifers of both breeds. The size differences are understandable, because dominant follicles are larger in ovulatory waves (Ali et al., 2001). In 3-wave cycles in C heifers we found significantly smaller DFs in the 2^{nd} wave compared to wave 1 or 3. In H heifers we found no differences. Nevertheless, statistical differences were determined in the group of all experimental animals.

The mean size of ovulatory follicles in 2-wave and 3-wave IOIs was 1.37 and 1.15 cm, respectively, in the group of all experimental animals. Similar differences were found when 2- and 3-wave IOIs in H and in C heifers were compared. These variations were statistically significant. Identical differences between the sizes of ovulatory follicles in 2- and 3-wave IOIs were described in Holstein heifers (Sirois and Fortune, 1988; Ginther et al., 1989; Sartori et al., 2004) and in Holstein lactating cows (Ginther et al., 1989; Townson 2002; Celik et al., 2005), but data on combined breeds of cattle are missing.

In conclusion, we found a similar pattern of ovarian follicular dynamics in Czech Fleckvieh and Holstein groups of heifers kept under identical conditions of nutrition and environment. The results obtained from Holstein heifers generally correspond to data published by other authors. Interestingly, the number of follicles per individual growth wave was lower, but this could be due to the methodological approach we chose. We demonstrated the influence of the number of follicular waves on the length of interovulatory intervals. Whether the significant difference in the emergence of the 1^{st} follicular wave in 2-wave IOIs between Czech Fleckvieh and Holstein heifers is of real biological significance is ambiguous. Variations in data presented in literature concerning the wave pattern of follicular growth per IOI can be due to its variability even in individual animals.

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