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RELATIONSHIP BETWEEN POSTPARTUM DISORDERS AND PREGNANCY LOSSES AND SUBSEQUENT FERTILITY OF DAIRY COWS

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Abstract

The aim of the study was to find the relationship between some postpartum disorders and pregnancy losses (PL), and the influence of PL on further fertility of Holstein-Friesian dairy cows. The 1,056 pregnancies and 906 calving intervals (CI) of 378 cows during the nine-year observation period were analysed. The average milk yield during this period increased from 7,500 to 10,500 kg/305 d of lactation. All cows up to 4 weeks after calving were gynecologically examined and cases of genital tract disorders were recorded and treated. Artificial inseminations (AI) were made using commercial frozen/thawed semen. In cows with silent ovulation, oestrus was induced by injection of $PGF_2\alpha$ or programmes using $PGF_2\alpha$ and GnRH, or hCG were applied. Pregnancy was diagnosed with the use of ultrasonography or foetal membrane slip only from 30 and 42 d after service, respectively, and re-diagnosed between 70 and 90 d after AI. The cases of „early foetal loss" up to 90 d and „mid-to-late PL" between 91 and 260 d after AI were recorded. Endometritis was noted in 31.8%, ovarian cysts in 8.5%, whereas ovarian inactivity in 4.6% of cows. Early PL was observed in 5.2%, whereas mid-to-late PL in 6.8% of cows (total 12%). From all analysed factors, the influence of delayed or periodical lack of ovarian activity was significant with regard to early PL (P=0.029). The probability of early PL increased when besides the diagnosis of ovarian inactivity, AI was made following an induced oestrus (P=0.003). CI for cows, which did not lost pregnancy amounted 442.9 d. The early, mid-to-late and multiple PL extended the CI to 579.6, 661.1 and 657.7 d, respectively (P<0.05). Concurrently, CI for cows with "early foetal loss" was significantly shorter comparing to cows with "mid-to-late foetal loss" (579.6 d *vs.*661.1 d; P<0.05). On average 2.6 inseminations for one pregnancy were needed, and each PL increased the AI index. Significant differences were stated after early and multiple foetal losses (5.2 and 5.1 *vs.* 2.6; P<0.05).

Key words: cows, pregnancy loss, reproduction disorders, fertility.

The influence of postpartum diseases on dairy cow's reproductive performance was presented in numerous studies. Pronounced deterioration in fertility indices was connected with retention of foetal membranes and puerperal metritis (3, 7, 13). The further consequences of these disorders are clinical and subclinical endometritis (8, 10, 16, 17). They extend the time from calving to the first ovulation and cyclic ovarian activity (27, 30, 31, 35). Cows with prolonged luteal phase or anovulatory cycle became pregnant at a lower rate and later comparing to cows with normal resumption of ovarian postpartum cyclicity (9, 32). Delayed resumption of ovarian activity, anoestrus, and cystic ovaries were associated with an increase in days to the first service, a decreased conception rate at the first service, and an extension of the days to conception (7, 11, 18, 22). Endometritis delays the time to the first (9) and successful artificial insemination (AI) (1, 10), increases the AI index (1), and increases the risk of culling cows because of infertility (3). The lack of possibilities to adequate balance of the ration or the energy deficiency causes an excessive loss of body condition that in consequence contributes to the disorders in ovarian function (3, 31, 34, 37). Cows in severe negative energy balance reveal histological evidence of higher degree of inflammation within the endometrium (36).

Many authors constantly report that as many as 9%-12% of dairy cows diagnosed pregnant at 30–45 d do not calve to the breeding responsible for the diagnosed pregnancy (6, 33, 38). Foetal attrition varies considerably from region to region, and with type and age of animal (dairy or beef, lactating or dry, heifer or cow), and to some extent, between intensively and extensively raised cattle (4, 12). The list of the factors having a negative impact on embryo/foetus is wide. They can be grouped and divided into: physiological, metabolic/nutritional, environmental, infectious, and management, but in practice pregnancy loss (PL) very often is the effect of sum of all or some of them.

The aim of the study was an attempt to find the relationship between some postpartum disorders and pregnancy losses, and the influence of pregnancy loss on further fertility of cows.

Material and Methods

The study included the occurrence of PL, diseases, and reproduction disorders of post-natal period and fertility indices of 378 Holstein-Friesian cows from the same dairy herd. The herd was free from contagious bovine abortions. The 1,056 pregnancies and 906 calving intervals during the nine-year observation period were analysed. Average milk yield during this period increased from 7,500 to 10,500 kg/305 d of lactation. The animals were kept in free stall barns. A total mixed ration (TMR) was provided twice a day. Routine reproductive tract examinations (based on vaginal and rectal palpation) were performed up to four weeks after calving. Cows after abortions, heavy parturitions, twin pregnancies, retained foetal membranes, as well as cows, which normally calved but showed clinical metritis or endometritis were treated systemically and/or locally. Inflammations of the uterus were classified on the basis of characteristics of the discharge obtained during the examination *per vaginam* and corresponding to the definition suggested by Sheldon *et al.* (29): healthy uterus - clear mucus, E1–clinical endometritis (mucopurulent discharge with the prevalence of mucus), E2–clinical endometritis (purulent discharge, >50% pus), and clinical metritis (fetid, purulent discharge, and enlarged uterus without general sings of illness). Ovarian cysts (OC) are defined as an anovulatory fluid-filled follicle-like structures of the ovary, >24 mm in diameter, which persist on the ovary for more than 7-10 d (26). Delayed resumption of ovarian activity was defined according to Gautam *et al.* (11) as occurring first ovulation beyond 35 d postpartum. Ovarian inactivity was defined based on the absence of the corpus luteum, in two consecutive examinations with 2 weeks interval, after 35 d p.p. This definition corresponds to types I and II classification of anestrous conditions based on the morphology and physiology of ovarian follicles in cattle, presented by Peter *et al.* (26). The cow was considered as a repeat breeder (RB) if she had at least 3 AI and no subsequent pregnancy. Cows treated at least once since the start of the service period for the following chronic genital tract diseases: cystic ovaries, anoestrous, suboestrous, endometritis, and pyometra were not considered as RB as suggested by Gustafsson *et al.* (14). In cows with silent ovulation (ovulation without signs of oestrus, corpus luteum palpated *per rectum*) oestrous was induced by injection of natural (dinoprost) or synthetic (cloprostenol) prostaglandin or using programmes combining injections of prostaglandin with GnRH (OvSynch) or hCG. Artificial inseminations with commercial, frozen/thawed semen were made by an AI technician in spontaneous or stimulated oestrus. Pregnancy examinations were performed *per rectum*, using an ultrasound scanner - model 480 (Pie Medical, the Netherlands) or by foetal membrane slip test only, from 30 and 42 d after the service, respectively. The first pregnancy examinations were made not later than 55 d post AI. Pregnancy was re-diagnosed between 70 and 90 d after service. Pregnancies, diagnosed at first examination but not confirmed at control checking, were classified as „early foetal loss". PL were also noted later between 91 and 260 d after AI. Diagnoses of these cases based on information concerning abortion and additional examinations of pregnant cows (according to earlier examination), in which signs of oestrus or pathological discharge from the vagina, were observed. No symptoms of coming parturition at the predicted time were the reason for the re-control pregnancy examination. These cases created the group of cows with „mid-to-late foetal loss". Cows that lost pregnancy close to 260 d, in dry period, most frequently started the new lactation. This group of cows was defined as a „mid-to-late foetal loss"/new lactation. Cows with two or three consecutive PL, in varied time of its development, during the one lactation, created the group with "multiple foetal losses".

The data were analysed by logistic regression, χ^2 test and ANOVA using the STATISTICA StatSoft®-PL programme. The Wald χ^2 statistic was used to determine the significance of each analysed variable effect in the logistic regression model on PL. For each variable, the adjusted odds ratio (AOR) were estimated. Comparison of method of the oestrus induction on PL was done using the χ^2 test. Analysis of variance (ANOVA) was implemented to compare calving interval, calving conception period, and insemination index among groups without foetal loss, with „early", „mid-to-late", and „multiple" foetal losses. Comparisons between means were performed by Student-Newman-Keuls multiple range test. In all tests effect was considered to be significant at $P<0.05$.

Results

The occurrence of reproductive tract disorders in the examined population of cows is presented in the Table 1.

It is clearly visible that endometritis was the main postpartum disease in the analysed cows. The main reproductive disorder was the repeated breeding. Ovary cysts in period preceding pregnancy were diagnosed in 8.5%, whereas problem with ovarian activity occurred in 4.6% of cows. Two thirds of the examined cows became pregnant after 120 d from calving and every fourth pregnancy was established after insemination following an induced oestrus.

Pregnancy losses were observed in 127 cases independently on the lack of genital tract pathology or occurrence the post-calving diseases and disorders. The early PL was noted in 5.2%, whereas mid-to-late foetal loss in 6.8% cows. This pathological condition relatively more often occurred in cows early exhibiting clinical endometritis, clinical metritis, and ovarian inactivity.

From all analysed factors (Table 2) the influence of delayed or periodical lack of ovarian activity was significant with regard to early PL ($P=0.029$). The multi–factorial analysis the interactions between the reproduction diseases and disorders and PL showed that the probability of the early pregnancy interruption increased when apart from the statement of ovarian inactivity during the puerperal period, the AI was performed following an induced oestrus ($P=0.003$).

Table 1
Characteristics of some diseases and disorders during the postpartum period preceding pregnancy and PL.

Analysed factors	Total cases n (%)	Pregnancy losses		
		Early n (%)	Mid-to-late n (%)	Together n (%)
Pregnancies	1,056 (100)	55 (5.2)	72 (6.8)	127 (12.0)
Clinical endometritis - E1	238 (22.5)	15 (6.3)	14 (5.9)	29 (12.2)
Clinical endometritis - E2	71 (6.7)	6 (8.4)	6 (8.4)	12 (16.9)
[2] Clinical metritis	27 (2.6)	-	4 (14.8)	4 (14.8)
Together	336 (31.8)	21 (6.3)	24 (7.1)	45 (13.4)
Ovarian cysts	90 (8.5)	3 (3.3)	6 (6.7)	9 (10.0)
Ovarian inactivity	49 (4.6)	6 (12.2)	2 (4.1)	8 (16.3)
Cows without diseases	565 (53.6)	24 (4.2)	39 (6.9)	63 (11.1)
Repeated breeding	282 (26.7)	18 (6.4)	19 (6.7)	37 (13.1)
Cows with CI >120 d	697 (66.0)	37 (5.3)	49 (7.0)	86 (12.3)
Pregnancies after induced oestrus	268 (25.4)	16 (5.9)	20 (7.5)	36 (13.4)

[1] data represent the number of foetal loss and (number of foetal loss cases/total number of PL in the group) ×100%; [2] there were not stated cases of clinical metritis in cows affected with PL to 90 d after insemination.

Table 2
Influence of the analysed factors on pregnancy losses

Interactions	Early foetal loss			Mid-to-late foetal loss		
	Wald χ^2 statistic	[1]AOR	P-value	Wald χ^2 statistic	[1]AOR	P-value
Endometritis (total):	1.1529	1.356	0.283	0.1063	1.088	0.744
Clinical endometritis - E1	0.9524	1.368	0.329	0.1386	0.889	0.710
Clinical endometritis - E2	0.8094	1.251	0.368	0.4366	1.162	0.509
[2] Clinical metritis	-	-	-	2.0927	1.312	0.148
Ovarian cyst	0.6869	0.606	0.407	0.0131	0.952	0.909
Ovarian inactivity	4.7650	2.728	0.029	0.4267	0.619	0.514
Repeated breeding	1.0682	1.358	0.301	0.0000	1.001	0.997
CI >120 d	0.0439	1.062	0.834	0.1519	1.109	0.697
Cows with AI in induced estrus	0.2683	1.176	0.604	0.0465	1.063	0.829

[1] adjusted odds ratio; [2] no cases of clinical metritis were found in cows affected with early foetal loss

Table 3
Descriptive statistics for the method of oestrus induction after which the pregnancy was established and pregnancy loss cases.

Methods	[1]Pregnancy loss		Analysed pregnancies
	Early (n)	Mid-to-late (n)	
	55	72	1056
$PGF_{2\alpha}$:			
Cloprostenol	10 (18.2%)	10 (13.9%)	150
Dinoprost	3 (5.4%)	5 (6.9%)	69
Together	13 (23.6%)	15 (20.8%)	219
[2]Antibacterial drugs + $PGF_{2\alpha}$	1 (1.8%)	2 (2.8%)	14
[3] Others	2 (3.6%)	3 (4.2%)	35

[1] data represent the number of foetal losses (number of foetal loss cases/total number of PL in the group ×100%); [2] cephapirin, ampicillin, and neomycin, 2% solution of iodium and kalium iodatum; [3] OvSynch, hCG and $PGF_{2\alpha}$, GnRH and $PGF_{2\alpha}$.

Table 4
Some fertility indices of cows with pregnancy losses *versus* remaining cows in the herd.

Item	Lactations				
	without foetal loss	with „early foetal loss"	with „mid-to-late foetal loss"	with „mid-to-late foetal loss"/new lactation	with „multiple foetal losses"
n	828	32	19	23	4
Calving interval (d)	442.9±85.5[a]	579.6±107.0[b]	661.1±103.6[c]	395.5±56.9[a]	657.7±227.1[bc]
Calving conception period (d)	164.1±85.7[a]	316.7±108.4[b]	380.1±100.2[c]	154.2±59.1[a]	462.2±165.9[bc]
Insemination index	2.6±2.0[a]	5.2±3.1[b]	4.2±2.2[ab]	2.5±1.77[a]	5.1±1.8[b]

Values with different letters (a, b, and c) in rows differ significantly (P<0.05). The length of pregnancy in the analysed herd was 279.8 ± 4.8 d.

Table 3 shows the descriptive statistics with regard to the method of oestrus induction, used in reproduction management, after which pregnancy was established and pregnancy loss cases.

Most pregnancies after inseminations following an induced oestrus were established after an intramuscular injection of synthetic or natural prostaglandin $F_2\alpha$: 14.2% and 6.5%, respectively. A contribution of the other method of oestrus induction (see commentary for Table 3) to the establishment of pregnancies was much smaller (4.6% analysed pregnancies). From 219 pregnancies, established after prostaglandin injection, 13 (5.9%) were lost up to 90 d after AI, 15 (6.8%) were lost later, concurrently the lost pregnancies constituted 23.6% and 20.8% of the total number of early and mid-to-late foetal loss, respectively. The proportion of losses within each group supported the disadvantage of synthetic prostaglandin (18.2% *vs*. 5.4% in group with early foetal loss and 13.9% *vs*. 6.9% in group with mid-to-late foetal loss). A direct comparison did not indicate the influence of the type of prostaglandin on PL (P=0.848).

Fertility indices in cows with normal and interrupted pregnancy are presented in Table 4. Calving interval for cows with normal pregnancy amounted 442.9 d. Early abortion, mid-to-late foetal loss, and multiple PL, together with the new attempts of insemination, significantly extended calving interval (P<0.05). Concurrently, CI for cows with "early foetal loss" was significantly shorter than for cows with "mid-to-late foetal loss" (579.6d *vs*.661.1d; P<0.05). In order to achieve the pregnancy, on average 2.6 inseminations were needed. Each premature termination of pregnancy increased the insemination index. Statistically significant differences were stated after early and multiple (in one lactation) foetal losses (5.2 and 5.1 *vs*. 2.6; P<0.05).

Discussion

Different grade of clinical endometritis affected 31.8% of the examined cows. Other authors (1, 3, 10, 17) reported up to 23.6% cases of endometritis in particular herds. In parallel, in the period preceding pregnancy, ovarian cyst were found in 8.5%, whereas ovarian inactivity in 4.6% of cows. Rhodes *et al.* (28) indicated that between 11% and 38% of cows were anovulatory by 50-60 d postpartum, whereas 13%-43% of cows in pasture-based systems were anovulatory prior to the beginning of the breeding season. Fertility was low even in cows that ovulated following a period of anovulation or anestrus.

The main reproductive disorder in our examination was the repeated breeding, diagnosed in 26.7% of cows. The percentage of cows with this kind of disorder ranged from 10.1% to 36.8% (2, 10, 14). In the study of Moss *et al.* (25), the risk of repeated breeding increased in cows with puerperal and chronic metritis, cystic ovarian disease, stillbirth, with increasing days taken to reach peak milk yield, and in cows, which were sub-fertile in the previous lactation. A relatively high percentage of cows with endometritis and repeated breeding noted in our study can be an explanation to the delayed moment to AI and pregnancy.

It was found that 12% of cows lost their pregnancy. The early PL was noted in 5.2%, whereas mid-to-late foetal loss in 6.8% cows. Other authors reported PL from 7.6% to 13.0%, depending on the herd (6, 33, 38). Between 27 and 41 d after AI, 18% of the losses were stated and 6.4% between 6 and 22 week, with a decreasing tendency during the first and second trimester and renewed increasing between 231 and 260 d (6, 23, 24).

The effects of different puerperal diseases and disorders on PL were not frequently analysed. Lopez–Gatius *et al.* (20) demonstrated data relating to cows with retained placenta and pyometra, which were 1.8 and 2.6 times more inclined to abortion, respectively, than cows free from these diseases.

On the basis of the conducted examination it can be noticed that endometritis and ovarian cysts did not have significant effect on PL. From all analysed factors, the influence of delayed or periodical lack of ovarian activity was significant with regard to early pregnancy attrition. McDougall *et al.* (23) reported a higher PL in cows that were treated because of anoestrus. The lactating females with increased days, open the experienced greater early and mid-to-late foetal loss (15). Nevertheless, higher PL rate was also observed in cows inseminated during the two first months after calving (23). That can be connected with disorders of ovarian cycles and progesterone profiles (11, 31, 34). The scale of pregnancy attrition connected with ovarian afunction, despite statistical significance, seems to be, at least in our case, marginal (eight cows with ovarian afunction lost pregnancy) in comparison, for instance, with the cows in which no diseases and disorders of reproduction system were noted and which lost pregnancy (63 cases).

Our examinations, alike to Lopez-Gatius *et al.* (21) did not reveal the influence of the oestrous induction on PL. However, induction of oestrous in connection to a previously diagnosed anovulatory anoestrous, increased the risk of PL. Despite the fact, that total PL, both early and late, after the use of prostaglandin were similar, the proportion of losses within each of the group supported the synthetic prostaglandin disadvantage. Direct comparison did not show the impact of the type of prostaglandin on the foetal loss.

Early abortion, mid-to-late foetal loss, multiple foetal losses, and new attempts of insemination significantly extended the average calving interval and increased the insemination index. Statistically significant differences in our study were found after the early and multiple PL. In the study by Lee and Kim (19), the overall mean intervals from the abortion to the first service and conception were 63.4 and 101.8 d, respectively, and the mean interval from PL to the first service lasted longer in cows with pregnancy interruption during the third trimester comparing to females with PL during the first and second trimesters. As negative consequences of the PL retention of foetal membranes, endometritis and decreasing milk yield for 305 d of lactation were also described (5, 19).

Cows in which the termination of pregnancy was recorded close to 260 d, most frequently started the new lactation in dry period. This group of cows was defined as a „mid-to-late foetal loss"/new lactation. To this group may belong cows with accurate length of pregnancy and „premature calving" probably because of mistakes in insemination date records and/or cows, which were incorrectly inseminated, according to behavioural sings similar to oestrous (suggesting repeat oestrous) meanwhile, pregnancy was established after previous inseminations. Fertility indices for these cows were similar to indices for cows with mature calving.

In conclusion, the presented results indicate that some puerperal diseases as an ovarian inactivity and clinical endomertitis and metritis tended to increase PL in dairy cows. PL significantly extended calving interval and insemination index, especially after the early and multiple foetal losses.

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