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INCIDENCE, EFFECT ON REPRODUCTION AND TREATMENT POSSIBILITIES OF OVARIAN ABNORMALITIES IN LARGE-SCALE DAIRY CATTLE HERDS

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1. ANTECEDENTS AND OBJECTIVES OF THE RESEARCH

In the past decades, the dairy cow population under milk production recording decreased substantially in Hungary. Parallel to that, owing to the genetic progress the quantity of milk produced by cows specialised for milk production strikingly increased. However, simultaneously with the increase of the specific milk yield the reproductive problems causing severe economic losses have also become more common.

The progress of diagnostic methods and tools has made it possible to diagnose and prevent these problems. Of the devices used for that purpose, various types of ultrasound equipment have gained widespread use in the animal reproduction practice. In addition to facilitating early pregnancy diagnosis and the monitoring of embryonic development, these devices also make it possible to screen animals for numerous abnormalities and diseases affecting the uterus and ovaries.

Our studies conducted over the last few years have frequently demonstrated ovarian abnormalities adversely affecting fertility. The majority of these abnormalities are caused by luteal ovarian cysts (corpus luteum with a cavity, corpus luteum and lutein cysts). These structures can be diagnosed easily with the help of an ultrasound machine. Their early detection and rational treatment may improve the reproductive indices, thus reducing the related economic losses.

The biological bases of efficient and competitive milk production are available, and only the proper balance has to be found to ensure that the specialists in charge of dairy farms pay more attention to animal health including reproductive care, in addition to the necessary technical background as well as the nutrition and management technology meeting the animals' demands.

In this work I studied the incidence of luteal ovarian structures appearing on the ovaries of dairy cows, their effect on reproductive processes and the possibilities of their treatment. I conducted the research in the herds of large-scale Holstein-Friesian dairy farms operating in Hungary.

My efforts were prompted by my professional background, i.e. that since 2005 I have been working at two dairy farms of an agro-industrial share company first as an inseminator and then as breeding manager. During my everyday work I often encountered reproductive problems causing severe economic losses. A substantial part of these problems are caused by cystic alterations of the ovaries.

By transrectal ultrasound examination, a method that I used in the farm practice on a daily basis, a two-dimensional image of ovarian structures having unusual lutein content can be obtained and the formation and evolution of these structures can be studied easily. This makes it possible to study the developmental stages of the corpus luteum (CL) developing during the normal oestrous cycle and, parallel to that, the development and effect of any luteal structures that may appear.

The main objectives of my research were as follow:

1. In addition to monitoring the developmental stages of the CL formed during the normal oestrous cycle, I wanted to study
    - the incidence and development of any luteal structures other than the CL, possibly appearing on the ovaries in the postpartum period and after service, and
    - the effect of such structures on the conception rate
2. To determine the treatment possibilities of luteal ovarian structures

3. To monitor the normal (hormonally not influenced) oestrous cycle by transrectal ultrasonographic examinations and metabolic tests, with special regard to the occurrence of ovarian abnormalities and their effect on fertility
4. To use transrectal ultrasonography for continuously monitoring the reproductive status of cows that had calved 90–120 days previously and were inseminated once; to determine the relationship of reproductive status with milk production and metabolic status, with special regard to the occurrence of ovarian abnormalities and their effects on fertility

2. MATERIALS AND METHODS

I performed the practical part of the research on two specialised Holstein-Friesian dairy farms of an agro-industrial share company operating in Hungary, between January 2008 and October 2009.

2.1. Transrectal ultrasonography

I performed the transrectal ultrasonographic examinations integrated into the reproductive practice of the farms, using a portable (3 kg) EASI-SCAN bovine ultrasound scanner provided with a high-resolution, dual-frequency (4.5–8.5 MHz) linear probe and an ultrasound goggle (BCF Technologies, Great Britain).

2.2. Study of the incidence of luteal structures in the postpartum period and after service

In the postpartum period I examined a total of 518 cows. In addition to diagnosing retained placenta and the subsequent possible development of metritis, I determined the time of onset of follicular growth waves, the resumption of cyclic ovarian activity, the occurrence of the first ovulation and the incidence of any luteal ovarian structures (corpora lutea with cavity, corpus luteum cysts, lutein cysts) possibly occurring.

In the period after service 462 cows were subjected to ultrasonographic pregnancy testing 28–35 days after service. In cows found to be pregnant, I checked the presence of the CL and the incidence of CL with cavity occurring during an existing pregnancy. In non-pregnant cows, if they were cycling, I induced another oestrus by administering a prostaglandin product (Dinolytic 5 ml/injection, a total of 25 mg dinoprost) as a single intramuscular injection. In cows with non-cyclic ovarian function I applied the

Ovsynch protocol consisting of three intramuscular treatments to synchronise the ovulation and to start cyclic ovarian activity.

2.3. Treatment of luteal ovarian structures

2.3.1. Treatment of corpora lutea and corpora lutea with cavity with $PGF_{2\alpha}$

I treated a total of 217 CL of cycle and 171 CL with cavity with intramuscularly administered $PGF_{2\alpha}$ (2 ml Estrumate injection, a total of 500 μg cloprostenol) at the time of the ultrasound examination.

2.3.2. Treatment of follicular and luteal cysts with $PGF_{2\alpha}$

After diagnosing 18 follicular cysts and 124 luteal cysts, I applied a single intramuscular $PGF_{2\alpha}$ treatment (2 ml Estrumate injection, a total of 500 μg cloprostenol).

In the case of all ovarian structures (CL, CL with cavity, follicular and luteal cyst), the subsequent ultrasound check I compared the efficacy of the treatment administered against the given structure (i.e. determined whether complete luteolysis of the given structure had occurred or the structure had persisted), and subsequently I compared the number of cows inseminated and their conception rate.

2.4. Monitoring of the normal (hormonally not influenced) oestrous cycle

Ten cows were examined by transrectal ultrasonography once a week from postpartum day 35 to days 28–35 after service. On the day of the examination I recorded the milk production of the individual cows and took blood samples from all cows for further tests (determination of plasma NEFA, BHB and FRAP concentrations and serum carotene concentration). I centrifuged the blood samples at 2000 rpm for 10 minutes and stored the supernatant (serum or plasma) at –70 °C until assayed.

2.5. Continuous ultrasonographic and metabolic monitoring of cows that had calved 90–120 days previously and were inseminated once

At the time of ultrasound pregnancy testing performed on days 28–35 after the first service, the cows were divided into three groups:

- pregnant cows (n=6)
- non-pregnant cows with cyclic ovarian activity (n=10)
- non-pregnant cows with luteal ovarian structures (n=10)

2.5.1. Pregnant cows: at the time of pregnancy testing I recorded the given cow's body condition score (BCS) and milk production, and took blood samples for further tests (for determining the plasma concentrations of NEFA, BHB and FRAP as well as serum carotene concentration). On day 60 after service I checked whether the previously diagnosed pregnancy still existed.

2.5.2. Non-pregnant cows with cyclic ovarian activity: on the ovaries of cows in this group I found a physiological CL; therefore, after the blood sampling I treated these cows with a single intramuscular prostaglandin injection (Dinolytic 5 ml/injection, a total of 25 mg dinoprost), which is expected to induce oestrus within 2–5 days.

Subsequently, I performed a comprehensive ultrasound examination of the ovaries and uterus once a week, and recorded the milk production and the body condition scores. In addition, twice a week I took blood samples for the laboratory analysis of the metabolic parameters.

If the cow had come into oestrus and had been inseminated, an additional ultrasound examination and a blood sampling were performed in all cases. Observation and comprehensive examination of the cows were performed up to days 28–35 after service. If the cow returned to oestrus after service, I continued the examinations until the pregnancy test following the subsequent service. Sixty days after service I repeated the pregnancy testing of the pregnant cows.

2.5.3. Non-pregnant cows with luteal ovarian structures

In cows of this group, I detected luteal structures (CL with cavity and/or lutein cyst and/or CL cyst) on the ovaries. The treatment administered was identical with that described in the case of non-pregnant cows having cyclic ovarian activity (see paragraph 2.5.2). If the cow failed to come into oestrus within 2–5 days after a single prostaglandin (PG) treatment, I repeated the PG treatment after 10 days. If oestrus could not be observed after this treatment either, after further 10 days I applied the Ovsynch protocol consisting of three intramuscular injections.

2.6. Biochemical (metabolic) tests

Blood samples were always taken from the coccygeal vein of the animals, into blood collection tubes without an anticoagulant and into those containing an anticoagulant (EDTA). The following tests were performed:

- *measurement of the ferric reducing ability of plasma (FRAP)*
- *determination of the non-esterified fatty acid (NEFA) concentration*
- *determination of the beta-carotene concentration*
- *determination of the beta-hydroxybutyrate (BHB) concentration*

These tests were performed in the Laboratory of Physiology of the Research Institute for Animal Breeding and Nutrition and in the Laboratory of the

Department and Clinic of Reproduction, Faculty of Veterinary Science, Szent István University.

2.7. Feed analyses

In June 2008, we conducted a herd-level survey (involving 5–10 cows per production group) on both farms to determine blood plasma beta-carotene concentrations and thus to assess beta-carotene supply status.

On four occasions during the period of the experiments (on 26 September 2008, 21 November 2008, 17 February 2009 and 9 April 2009) we analysed total mixed ration (TMR) samples from the feeding trough in the dry, close-up, post-calving and high-producing cow groups. The TMR samples were assayed for original dry matter content, crude protein, ether extract, crude fibre, nitrogen-free extracts and carotene.

The analysis of blood and feed samples was conducted in the Laboratory of the Department of Nutrition of the Faculty of Agriculture and Environmental Sciences of Szent István University.

2.8. Statistical evaluation

2.8.1. In the postpartum period, I compared the incidence of metritis and irregular ovarian structures with data reported in the special literature. I used the Chi-square test to detect differences between cows affected with metritis and/or retained placenta and healthy cows in terms of the incidence of irregular luteal structures. The same method was used **in the period after service** to demonstrate differences between cows conceiving after a single service, those conceiving after multiple services and cows not inseminated in the test period in the number of irregular luteal structures appearing on the ovaries.

2.8.2. The effect of $PGF_{2\alpha}$ treatment on corpora lutea and corpora lutea with cavity, and efficacy of the treatment against follicular and lutein cysts (i.e. whether complete luteolysis of the given structure occurred or the structure persisted), the ratio of cows inseminated and the conception rates were evaluated by Chi-square test and Fisher's test.

2.8.3. When monitoring the normal (hormonally not influenced) oestrous cycle, after analysing the ultrasound scans I assigned a specific value to each ovarian structure of all the 10 cows, so that I could graphically represent the cycle curve.
To demonstrate possible differences in milk production and metabolic blood parameters between cows that failed to conceive and the conceived cows, I used the paired *t*-test and the Chi-square test.

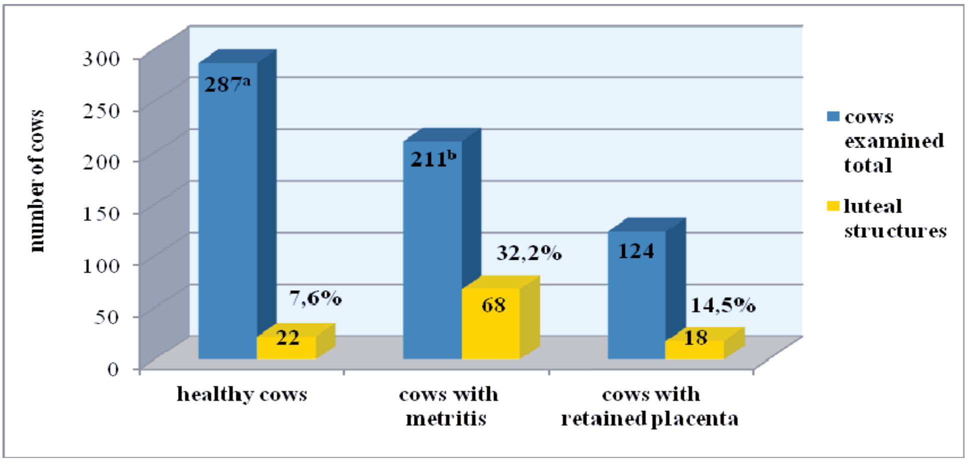
2.8.4. When analysing the results of continuous ultrasonographic and metabolic monitoring of cows that had calved 90–120 days previously and were inseminated once, I evaluated the correlations between the metabolic parameters and the success of inseminations by the Chi-square test. The same statistical method was used for detecting significant differences in milk production between cows having cyclic ovarian activity and those having irregular lutein cysts, and having conceived or failing to conceive during the period of the experiment.

3. RESULTS

3.1. Study of the incidence of luteal structures in the postpartum period and after service

3.1.1. Results of transrectal ultrasonography performed in the postpartum period

The herd-level surveys conducted by me demonstrated hat the incidence of metritis was 40.7% in the postpartum period. It can be established that, in addition to the high number of involution problems, cows affected with metritis showed a significantly ($P<0.001$) higher incidence of luteal ovarian structures than healthy cows (**Figure 1**).



There is a statistical difference between the quality groups with differents letters

Figure 1. *Incidence of luteal ovarian structures in the postpartum period*

3.1.2. Results of transrectal ultrasonography performed in the period after service

In the period after service, no significant difference was found in the incidence rate of luteal structures between cows conceiving after the first service and those requiring multiple services to conceive. In contrast, in cows that failed to conceive during the period of study the incidence rate of luteal ovarian structures was significantly ($P<0.001$) higher.

In 14 of the successfully inseminated cows (4.2%), pregnancy was maintained by a corpus luteum with cavity at the time of ultrasonographic pregnancy testing performed on days 28–32 after service. However, the control pregnancy testing carried out on day 60 after service demonstrated fetal loss in 43% of these cows. In the remaining 8 cases (57%), pregnancy was already maintained by a CL without a cavity. This may indicate that corpora lutea with a cavity have lower efficacy in maintaining pregnancy (i.e. they are not necessarily capable of performing their function); at the same time, the presence of a cavity does not rule out the maintenance of pregnancy either, as in the pregnant cows the cavity of the CL was found to disappear after some time.

3.1.3. Results of serum beta-carotene determinations and feed analyses performed in the postpartum period and after service

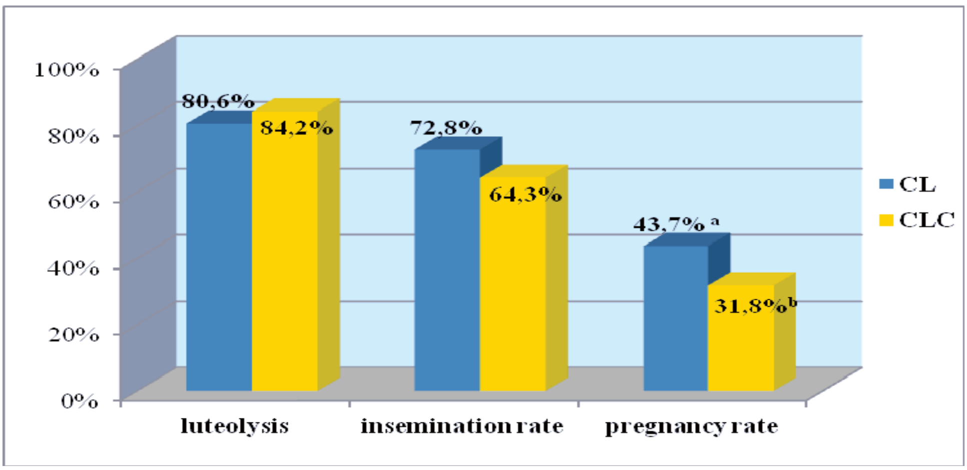
The high incidence of metritis developing in the postpartum period can be explained by the severe, herd-level beta-carotene deficiency detected by serum beta-carotene determinations in the postpartum period and after service, as well as by the beta-carotene deficiency demonstrated by the analysis of TMR samples from the feeding troughs in the prepartum (dry and close-up) cow groups. Namely, beta-carotene and vitamin A exert their effect mainly by stabilising the cell membrane. Stability of the cell membranes is important for hormone production of the ovaries, follicle maturation processes and regeneration – involution – of the uterine mucosa *(Mézes,2003).*

The feed analyses demonstrated, on herd level, crude protein values lower than the required level (16–18%, i.e. 160–180 g/kg dry matter), or values that were at the lower limit of the acceptable range. Herd-level protein deficiency may also increase the number of acyclic cows or cows having an inactive ovary *(Brydl and Gábor, 2005*).

3.2. Treatment of luteal structures occurring on the ovaries

3.2.1. Treatment of corpora lutea and corpora lutea with cavity with $PGF_{2\alpha}$

A single intramuscular $PGF_{2\alpha}$ injection may be used successfully for inducing luteolysis of corpora lutea with cavity, in the same way as in the case of corpora lutea originating from the normal cycle. However, I found a significantly ($P<0.05$) lower pregnancy rate among cows having a corpus luteum with cavity (**Figure 2**).



There is a statistical difference between the quality groups with differents letters

Figure 2. *Effect of a single $PGF_{2\alpha}$ treatment on the pregnancy rate*

3.2.2. Treatment of follicular and luteal cysts with $PGF_{2\alpha}$

When we administered a single intramuscular $PGF_{2\alpha}$ injection to treat follicular and luteal cysts, we observed that although luteolysis occurred in many cases also in the group of cows having follicular cysts, its rate was significantly ($P<0.05$) higher in the group of cows having luteal cysts. As the majority of cows in both groups responded to PG treatment, it is likely that not only the luteal structure contained lutein cells (**Table 1**).

Table 1. *Results of a single $PGF_{2\alpha}$ treatment of follicular (FC) and luteal cysts (LC)*

PARAMETER	FC		LC	
	n	%	n	%
Total number examined	**18**		**124**	
Persistence	**6**	**33.3**	**5**	**4**
Luteolysis within 5 days	**12**	**66.7**	**119**	**96**
Inseminated	**9**	**50**	**51**	**42.9**
Pregnant	**6**	**66.7***	**26**	**51***
Non-pregnant	3	33.3*	25	49*
Not inseminated	**9**	**50**	**68**	**57.1**

*as a proportion of the cows inseminated

3.3. Monitoring of the normal (hormonally not influenced) oestrous cycle

3.3.1. Results of ultrasonographic cycle diagnosis

Seventy percent of the cows examined had cyclic ovarian activity at the start of the study (I detected a corpus luteum on at least one of their two ovaries). At the same time, in 20% of the cows a CL characteristic of the cycle appeared by postpartum day 45, while in one cow this occurred only on postpartum day 79 (**Figure 3**).

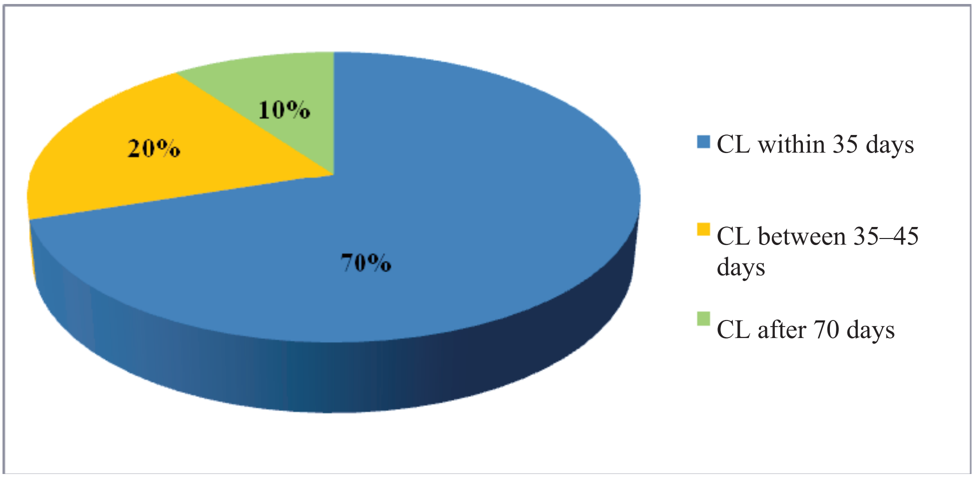
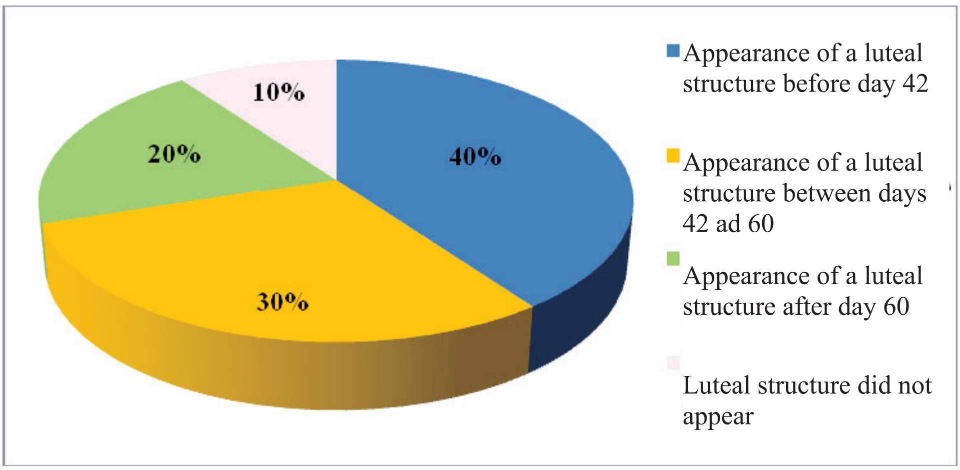


Figure 3. *Resumption of cyclic ovarian activity after calving*

Luteal structures appeared by postpartum day 42 in 40% of the cows, by postpartum day 60 in further 30% and only after postpartum day 60 in 20% of the cows (**Fig. 4**). This period has outstanding importance because of the possible development of infertility, as in cows having a healthy oestrous cycle this period is the time of oestrus manifesting itself in clear clinical signs.



Fiure 4. *Time of appearance of luteal ovarian structures after calving*

3.3.2. Visual analysis of the cycle curves

Based upon visual analysis of the cycle curves it can be established that those cows (n=4) conceived during the period of the experiment in which at least one of the two ovaries continuously showed cyclic activity and in which luteal ovarian structures (corpus luteum with cavity, corpus luteum cyst) were diagnosed only once or not at all. Cows in which an irregular lutein structure was diagnosed on the ovaries on multiple occasions and/or continuously for a varying length of time and, in addition, one of the ovaries was inactive or there was no regular cyclic ovarian activity, did not conceive during the period of study.

3.3.3. Correlation of the success of fertilisation with milk production and the results of metabolic tests

Conception did not take place in cows in which beta-carotene concentration of the blood serum remained below 3 μmol/l throughout the experimental period. At the same time, cows with an average serum beta-carotene concentration above the upper limit of the physiological range showed a pregnancy rate of 50%.

The fact that the average value of FRAP, an indicator of blood plasma antioxidant capacity, was below the 150 μmol/l physiological limit in a significantly higher proportion (17.4%) of cows failing to conceive during the experimental period is not surprising, as this had already been predicted by the result of the herd-level beta-carotene survey. Therefore, this could also have contributed to the increased incidence of involution disturbances.

In samples from the two cows with the highest milk production (38.5 and 39.1 kg), the measured NEFA concentrations exceeded the limit by 35–43%. By analysing the cycle curves of these cows it can be seen that one of them did not ovulate again for a relatively long time (60 days), while in the case of the other cow I diagnosed luteal ovarian structures (corpus luteum with cavity, corpus luteum cyst) on multiple occasions (**Tables 2a and b**).

Table 2. *Milk production and metabolic test results of cows examined during a normal (hormonally not influenced) oestrous cycle*

Table 2a. *Test results of cows that conceived during the experimental period*

EAR-TAG NUMBER	MILK	NEFA	BHB	FRAP	CAROTENE
	mean (kg)	**mean (mmol/l)**	**mean (mmol/l)**	**mean (µmol/l)**	**mean (µmol/l)**
1863	33.9	0.245	0.173	220.16	6.6
2962	28.6	0.156	0.136	212.37	3.0
3300	20.5	0.16	0.218	231.42	3.8
617	31.3	0.228	0.136	196.57	6.8
MEAN	**28.6**	**0.197**	**0.166**	**215.13**	**5.05**
STANDARD DEVIATION	**0.6**	**0.046**	**0.039**	**14.63**	**1.93**

Table 2b. *Test results of cows that failed to conceive during the experimental period*

EAR-TAG NUMBER	MILK	NEFA	BHB	FRAP	CAROTENE
	mean (kg)	**mean (mmol/l)**	**mean (mmol/l)**	**mean (µmol/l)**	**mean (µmol/l)**
1752	29.7	0.14	0.376	231.42	1.8
2077	39.1	0.245	0.128	165.625	7.6
2920	38.5	0.26	0.126	222.89	2.6
423	24.9	0.11	0.081	200.71	5
5045	29.4	0.12	0.232	241.46	5
951	29	0.17	0.237	206.81	5.2
MEAN	**31.8**	**0.174**	**0.197**	**211.49**	**4.53**
STANDARD DEVIATION	**5.7**	**0.064**	**0.107**	**27.081**	**2.07**

3.4. Results of ultrasonographic monitoring and metabolic tests of cows that had calved 90–120 days previously and were inseminated once

3.4.1. Visual analysis of the cycle curves

From the analysis of the cycle curves it can be established that prostaglandin treatment (Dinolytic 5 ml intramuscular injection, a total of 25 mg dinoprost) administered at the beginning of the study resulted in luteolysis of the structure in 50% of the cows in both the group designated *Non-pregnant cows with cyclic ovarian activity* and in that designated *Cows with luteal ovarian structures*. During the experimental period, 40% of the cows in the group 'non-pregnant cows with cyclic ovarian activity' and 50% of the cows in the 'cows with a luteal ovarian structure' could be fertilised successfully. Significant difference could not be demonstrated between the two groups.

3.4.2. Correlations of the success of insemination with milk production and metabolic test results

In the **pregnant cows,** none of the metabolic parameters deviated from the physiological limits, indicating that the parameters measured are probably good indicators of the metabolic status of pregnant animals (**Table 3**).

__Table 3.__ Results of metabolic tests in pregnant cows

EAR-TAG NUMBER	NEFA	BHB	FRAP	CAROTENE
	Average of 2 measurements (mmol/l)		Average of 2 measurements (μmol/l)	
1110	0.522*	0.075	312	10.93
4232	0.295	0.075	292	10.65
3430	0.095	0.08	242	3.96
235	0.191	0.1	538	6.98
417	0.081	0.065	360	7.01
4163	0.058	0.08	333	8.5
MEAN	**0.207**	**0.079**	**346.17**	**8.01**
SD	**0.098**	**0.012**	**112.601**	**2.45**

* the result of only one of the measurements (0.691 mmol/l) exceeded the physiological limit of 0.4 mmol/l

The values of metabolic parameters measured in the group of **non-pregnant cows having cyclic ovarian activity** may also serve as indicators. The serum beta-carotene concentration of cows that conceived in this group did not drop below the limit in any of the cases, and the same holds true for the plasma FRAP values. This seems to confirm the hypothesis that serum beta-carotene concentrations and plasma FRAP values reaching or exceeding the physiological limit values are associated with higher fertility rates. No statistically significant differences could be observed between the conceived and non-conceived cows in the plasma concentrations of BHB and NEFA, which are indicators of energy supply (**Tables 4a and 4b**).

Table 4. *Milk production and metabolic test results of non-pregnant cows having cyclic ovarian activity*

Table 4a. *Test results of cows that became pregnant during the experimental period*

EAR-TAG NUMBER	MILK	NEFA	BHB	FRAP	CAROTENE
	mean (kg)	**mean (mmol/l)**	**mean (mmol/l)**	**mean (µmol/l)**	**mean (µmol/l)**
1832	29.5	0.084	0.336	209	8
2195	31.2	0.295	0.172	251	4.34
2751	32.5	0.243	0.119	215	4.43
423	27.6	0.222	0.125	235	6.6
MEAN	**30.2**	**0.211**	**0.188**	**228**	**5.84**
SD	**2.1**	**0.090**	**0.101**	**19.2**	**1.77**

Table 4b. *Test results of cows that did not become pregnant during the experimental period*

EAR-TAG NUMBER	MILK	NEFA	BHB	FRAP	CAROTENE
	mean (kg)	**mean (mmol/l)**	**mean (mmol/l)**	**mean (µmol/l)**	**mean (µmol/l)**
1203	28	0.224	0.142	330.23	5.27
248	34.6	0.122	0.06	221.76	5.9
2639	28	0.125	0.115	214.53	3
3276	30.6	0.189	0.089	184.88	3.58
3319	24.2	0.056	0.166	224.92	1.74
3724	32.5	0.199	0.133	215.36	4.38
MEAN	**29.7**	**0.153**	**0.118**	**231.95**	**3.98**
SD	**3.7**	**0.062**	**0.038**	**50.21**	**1.52**

While it is true that in the group having a **luteal ovarian structure** FRAP values lower than the physiological limit occurred with the same frequency among the cows that had become pregnant and those that failed to conceive, in this group FRAP values lower than the physiological limit were found in a much higher proportion of the cows than in the cow group having cyclic ovarian activity (**Tables 5a and 5b**).

Table 5. *Milk production and metabolic test results of non-pregnant cows having a luteal ovarian structure*

Table 5a. *Test results of cows that became pregnant during the experimental period*

EAR-TAG NUMBER	MILK	NEFA	BHB	FRAP	CAROTENE
	mean (kg)	**mean (mmol/l)**	**mean (mmol/l)**	**mean (µmol/l)**	**mean (µmol/l)**
4352	36.7	0.136	0.123	326.25	7.8
5819	29	0.063	0.102	172.23	3.41
1921	28	0.16	0.234	237.41	7.04
4490	18.4	0.086	0.165	158.09	5.9
2761	19.6	0.092	0.165	189.5	5
MEAN	**26.3**	**0.107**	**0.158**	**216.696**	**5.83**
SD	**7.5**	**0.039**	**0.5**	**68.159**	**1.72**

Table 5b. *Test results of cows that did not become pregnant during the experimental period*

EAR-TAG NUMBER	MILK	NEFA	BHB	FRAP	CAROTENE
	mean (kg)	**mean (mmol/l)**	**mean (mmol/l)**	**mean (µmol/l)**	**mean (µmol/l)**
223	42.2	0.494	0.121	356.31	9.86
268	24.4	0.862	0.456	257.88	8.55
1852	39	0.331	0.03	216.29	4.33
561	33.3	0.118	0.087	202.12	7.97
2355	34.4	0.224	0.115	158.58	6.02
MEAN	**34.7**	**0.406**	**0.162**	**238.24**	**7.35**
SD	**6.76**	**0.29**	**0.168**	**74.93**	**2.18**

On the basis of the measurements it can be stated that those cows having a luteal structure failed to become pregnant during the period of experiment which had higher average milk production and blood plasma NEFA values that exceeded the normal limit value of 0.4 mmol/l on multiple occasions. There was a substantial difference in milk production between the non-pregnant and the conceived cows, and probably this explains the significant differences found in NEFA plasma concentrations between the two groups.

4. CONCLUSIONS AND RECOMMENDATIONS

Good reproduction is an essential condition of profitable milk production in dairy operations. Delayed conception after calving may lead to lower milk production. Parallel to the increase in milk production, the reproductive performance of dairy herds has notably decreased over the past decades. Disturbances of energy balance, delayed involution and the presence of luteal ovarian structures (corpus luteum with cavity, corpus luteum cyst, lutein cyst) associated with the absence of cyclic ovarian activity cause the most severe problems in the postpartum period.

The results of my herd-level surveys seem to corroborate the observation, described also by other researchers, that the incidence of metritis varies between 20% and 50% in the postpartum period (in the present study we measured an incidence of 40.7%). It can be established that, in addition to the high incidence of involution disturbances, the incidence of luteal ovarian structures is significantly ($P<0.001$) higher in cows with metritis than in healthy cows. Therefore, it would be worth paying closer attention to reducing the incidence of retained placenta and metritis in the postpartum period and to the soonest possible treatment of cows affected with such problems. By doing so, we can also reduce the incidence of luteal structures delaying the resumption of cyclic ovarian activity. The first two months of the postpartum period have outstanding importance from the point of view of infertility, as this is the period when oestrus manifesting itself in clear clinical signs occurs in cows with a normal cycle. However, when monitoring the normal (hormonally not influenced) oestrous cycle, I found that this is also one of the periods when the incidence of luteal structures reaches its peak (luteal structures appeared by postpartum day 42 in 40%, by postpartum day 60 in further 30% and only after postpartum day 60 in 20% of the cows).

Choosing the time of the first postpartum service also has outstanding importance. In my own experience, the highest pregnancy rate (66%) was achieved when cows were inseminated after postpartum day 120, while none of the cows inseminated before postpartum day 60 became pregnant. Fifty percent of the cows included in the study were inseminated between postpartum day 60 and 120, with 40% pregnancy rate. All these observations seem to corroborate the consistent opinion of experts dealing with dairy cow reproduction, i.e. that in high-producing dairy herds the optimum time of the first service is after postpartum day 60. Namely, by that time the postpartum negative energy balance of high-producing dairy cows is resolved and the histological involution of the uterus is completed. According to the decisive majority of researchers, the pregnancy rates resulting from service before postpartum day 60 are markedly lower than those achieved by inseminations carried out after postpartum day 60. It should be noted, however, that the desirable time of the first postpartum service of cows should be determined on the basis of not only biological but also economic considerations.

Examination of the inseminated cows revealed that luteal ovarian structures could be diagnosed in a significantly ($P<0.001$) higher proportion of the cows that had failed to conceive. A similar result was obtained when analysing the cycle curves: those cows became pregnant during the period of experiment in which at least one of the two ovaries continuously showed cyclic activity, and in which luteal ovarian structures (corpus luteum with cavity, corpus luteum cyst) were found only on a single occasion or not at all. Cows on the ovaries of which irregular lutein structures were diagnosed on multiple occasions and/or continuously over a varying length time and, in addition, one of their ovaries was inactive or did not have regular cyclic ovarian activity, did not become pregnant during the experimental period.

The results of my studies suggest that corpora lutea with cavity are less efficient in maintaining pregnancy than is the corpus luteum of pregnancy (i.e. the former are not necessarily capable of performing their function). The rate of fetal loss diagnosed by me was 43% in the case of corpora lutea with cavity and only 6.1% in the case of a normal corpus luteum of pregnancy. At the same time, the presence of the cavity does not exclude the maintenance of pregnancy, as according to my observations the cavity disappeared after a certain time in cows that remained pregnant.

The high incidence of metritis developing in the postpartum period can be explained by the severe, herd-level beta-carotene deficiency detected by serum beta-carotene determinations in the postpartum period and after service, as well as by the beta-carotene deficiency demonstrated by the analysis of TMR samples from the feeding troughs in the prepartum (dry and close-up) cow groups. Namely, beta-carotene and vitamin A exert their effect mainly by stabilising the cell membrane. Stability of the cell membranes is important for hormone production of the ovaries, follicle maturation processes and regeneration – involution – of the uterine mucosa. In addition, beta-carotene deficiency is known to result in delayed ovulation and delayed postpartum resumption of cyclic ovarian activity in cows.

The crude protein values found on herd level were lower than the requirement (16–18%, i.e. 160–180 g/kg dry matter) or were at the lower limit of the acceptable range. This is indicative of protein deficiency, which may also increase the number of acyclic cows or of cows having an inactive ovary.

The lower than 16% crude fibre content of the TMR, measured in the post-calving and the high-producing groups, poses the risk of acidosis, which is a metabolic problem potentially causing a further deterioration of the reproductive parameters. Thus, errors made in nutrition may generate animal health problems and reproductive disturbances, eventually decreasing milk production and causing severe economic losses.

The success of inseminations is also correlated with the results of metabolic tests: cows that had a blood serum beta-carotene concentration lower than 3 μmol/l throughout the experimental period did not become pregnant. On the other hand, cows having an average beta-carotene concentration above the physiological range showed 50% pregnancy rate.

FRAP concentrations below the limit of 150 μmol/l occurred in a significantly higher proportion (17.4%) of cows failing to become pregnant. This is not surprising, as it had been predicted by the results of earlier serum beta-carotene determinations. Therefore, this could also contribute to the increased incidence of involution disturbances. The continuous monitoring of cows that had calved 90–120 days previously and were inseminated once, it can be stated that in cows having cyclic ovarian activity and having become pregnant, the serum beta-carotene concentration and the plasma FRAP value never decreased below the limit value. All this suggests that this may be a possible reason why the pregnancy rate of cows having below-the-limit serum beta-carotene concentrations and plasma FRAP values was found to be lower. In cows having a luteal ovarian structure the incidence of below-the-limit FRAP values was higher than in cows with cyclic ovarian function.

Of the metabolic parameters, NEFA has an outstandingly important role, as indicated by the fact that in the two cows with the highest milk production I measured NEFA values exceeding the limit by 35–43% during the normal oestrous cycle. Analysis of the cycle curves of these two cows indicates that one of the two cows failed to ovulate again for a long period of time (60 days), whereas in the other cow I diagnosed luteal ovarian structures (corpus luteum with cavity, corpus luteum cyst) on multiple occasions.

Based on the plasma NEFA concentration measurements of cows that had calved 90–120 days previously and were inseminated once, it can be stated that those cows having a luteal structure failed to become pregnant during the period of the experiment which had a higher average milk production, and in which the serum NEFA concentration exceeded the physiological limit (0.4 mmol/l) on multiple occasions. There was a substantial difference in average daily milk production between the non-pregnant and the conceived cows, and probably this explains the significant differences found in NEFA plasma concentrations between the two groups. These results seem to confirm the observations reported in the literature that the high plasma concentration of NEFA inhibits the oestrogen production of follicles and the ovulation, and that due to the multiple cytotoxic effects of elevated NEFA concentration irregular (possibly lutein-containing) structures may be formed during the cycle.

All these findings underline the high importance of regular feed analyses and metabolic profile tests in the dairy herds.

In the same way as corpora lutea originating from the normal cycle, corpora lutea with a cavity may also be successfully luteolysed by a single intramuscular $PGF_{2\alpha}$ injection. However, the pregnancy rate is significantly ($P<0.05$) higher among cows having a corpus luteum of cycle than among animals having a corpus luteum with cavity. This suggests that the presence of a corpus luteum with cavity may cause lower fertility. It is difficult to differentiate animals in which the presence of the cavity has importance from those in which it is without significance; therefore, further investigations are needed to clarify this question. It is well known that the corpus luteum may have a cavity in the first days of the cycle, but this cavity usually disappears between day 6 and 8 of the cycle. Only cases in which this does not happen warrant further investigation.

After a single intramuscular $PGF_{2\alpha}$ treatment of structures (follicular and luteal cysts) selected on the basis of the currently most widely accepted categorisation (*Brito et al., 2004*) we found that luteolysis often occurred also in the group of cows having follicular cysts, but its rate was significantly higher in the group of cows having a luteal cyst. At the same time, the ratio of cows having become pregnant was significantly ($P<0.05$) higher in the group of cows having a follicular cyst. As the majority of cows in both groups responded to PG treatment, it is likely that not only the luteal cysts but also a certain part of the cysts categorised as follicular cysts contained luteal cells. This means that the earlier categorisation of the cysts is not perfect and needs refinement.

In conclusion, it can be stated that the incidence of luteal structures associated with the absence of cyclic ovarian function is correlated with the involution disturbances of the postpartum period, with the quantity of milk produced by the cows, with the nutritional deficiencies and also with the metabolic status of the animals.

Cows that have luteal ovarian structures on multiple occasions and/or for a longer period of time become pregnant later and with more difficulty. In the presence of corpora lutea with cavity diagnosed during pregnancy the risk of fetal loss is higher.

Therefore, it would be worth paying a closer attention to the correlations outlined above, which are indicators of one another, as they can allow us to draw important conclusions. Early detection and effective treatment can improve the reproductive indices and reduce the economic losses.

5. NEW SCIENTIFIC RESULTS

1. The incidence of luteal ovarian structures delaying the resumption of cyclic ovarian function is significantly ($P<0.001$) higher among cows affected with metritis than in healthy animals.
2. By the method of cycle curve analyses elaborated by me, I established that in cows failing to become pregnant the incidence of luteal ovarian structures is significantly ($P<0.001$) higher than in cows becoming pregnant after service.
3. Corpora lutea with cavity show lower efficiency in maintaining pregnancy, which is indicated also by the high rate of late fetal loss.
4. Beta-carotene and antioxidant deficiency as well as negative energy balance lowers the pregnancy rate of cows. High NEFA values indicative of negative energy balance are correlated with the occurrence of a higher number of luteal structures on the ovaries. If in cows with higher average milk production, parallel with the presence of luteal structures, the NEFA value exceeds the normal limit value of 0.4 mmol/l on multiple occasions, the cows will not become pregnant.
5. A single intramuscular $PGF_{2\alpha}$ treatment of corpora lutea originating from the normal cycle and corpora lutea with cavity can be similarly effective in inducing luteolysis of the structure; however, the pregnancy rate of cows having a corpus luteum with cavity is significantly ($P<0.05$) lower.
6. After a single intramuscular $PGF_{2\alpha}$ treatment of follicular and luteal cysts luteolysis often occurs also in the case of follicular cysts, suggesting that follicular cysts also contain lutein cells. This queries the accuracy of cyst categorisation.

6. PUBLICATIONS ON THE SUBJECT OF THE DISSERTATION

Scientific publications

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Presentations

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