## Asymmetrical functional activities of ovaries and tubular part of reproductive organs of dairy cows

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#### ABSTRACT

The aim of this study was to compare the effect of postpartal activity of the left and the right side of reproductive organs on subsequent fertility in dairy cows, depending on the side of previous gestation, after deep intracornual semen deposition, strictly in cows with single ovulations. The semen was deposited in the horn ipsilateral to the ovary with preovulatory follicle. The effects were studied in 2300 cows of Holstein-Friesian breed, out of which 937 (40.74%) were in their first lactation, 641 (27.87%) in their second, 417 (18.13%) in third and 305 (13.26%) in their fourth lactation. The first manifested oestrus occurred spontaneously in 2099 (91.26%) cows, while it was induced with the GPG (Ovsynch) method in 201 (8.74%) cows. The relationship between the sides of the previous gestation and the result of conception depending on the side of the ovulating ovary were compared. Significantly stronger right side activity of the reproductive organs was registered in the previous gestation: 1321 (57.43%) of pregnancies were in the right horn and 979 (42.57%) in the left horn. After the involution of the uterus, there was increased activity of the side opposite to the previous gestation, and mature follicles were found on the contralateral ovary in 1377 (59.87%) cows and on the ipsilateral ovary in 923 (40.13%) cows. The difference in the sides of ovulating ovaries was statistically significant, meaning that ovulations are more frequent on the ovary contralateral to the postpartal horn. These results significantly suggest that the bilateral asymmetry of reproductive organs has an influence on conception rates in dairy cows. The results also confirm asymmetry in the function of the reproductive organs in dairy cows, due to differences in ovarian activity and probably even more because of physiological differences in the tubular part of the

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reproductive organs, depending on the side of previous gestation and involution of the postpartal horn of the uterus.

Key words: dairy cattle, bilateral asymmetry, side of pregnancy, side of ovulation

#### Introduction

In embryonic development there should be equal activity of both sides of reproductive system (HUNTER, 1995), but there is much evidence of the influence of functional asymmetry on fertility parameters in older literature.

The percentage of right side ovulations is definitely higher than of left side ovulations. According to CLARK (1936), out of 704 gestations, only in 293 (42%) cows was it on the left side. NIELSEN (1949) found a significantly higher number of right side gestations. KIDDER et al. (1952) recorded 54.5% pregnancies on the right side. PERKINS et al. (1954) examined 1000 cows at a slaughter house, and found 25.5% (255) of them to be pregnant, and in 57.3% of cases it was a right sided pregnancy. REECE and TURNER (1954) examined 923 cows by rectal palpation on gestation, and found right side pregnancies in 62.5% (577) of cows. According to LOPEZ- GATIUS (1997) in 55.8% of cows the first pregnancy was on the right side.

STALFORS (1916) studied ovarian activity from 1907 to 1915, and found the right ovary to be more active than the left one. Ovaries on the right side are bigger and more active in cows (CASIDA et al., 1935; CLARK, 1936; SCHRAM, 1937; REECE and TURNER, 1954; ERDHEIM, 1942; SPRIGGS, 1945; CASIDA et al., 1948; NIELSEN, 1949; RAJAKOSKI, 1960; KIDDER et al., 1952; LAGERLOF and BOYD, 1953; PERKINS et al., 1954; MORROW et al., 1968; PIERSON and GINTER, 1987; SCHNEEBELI and DÖBELI, 1991), which might be an explanation for this phenomenon. According to CASIDA and VENZKE (1936) the time period needed for formation of mature follicle, ovulation and subsequent formation of corpus luteum (CL) was  $8.1 \pm 2.5$  days shorter in cows with ovulation on the contralateral ovary than in cows with ovulation on the ovary containing a CL from a previous gestation. According to BELLIN et al. (1984), follicles developed on ovaries containing CL from a previous gestation have a smaller diameter than those on ovaries without CL from a previous gestation. MATTON et al. (1981) considered that CL directly inhibits folliculogenesis, because there is a larger number of developing middlesize follicles during the whole oestrous cycle on ovaries without CL from a previous gestation compared to ovaries containing one. Corpus luteum has an influence on follicle diameter on days 8th to 13th, and increases the number of middle- size follicles in all phases of the oestrous cycle. KIDDER et al. (1952) analysed variability in the occurrence of ovulation in dairy cows depending on the side of the reproductive system, and tried to explain differences in ovarian activity, the right ovary being more active than the left one. Studying the first oestrus in heifers, authors have found that the first ovulation took place on the right ovary in 54.1% of heifers, the second in 59.6% of them, with an average of 56.5% of ovulations being on the right side. As an example of asymmetry, CASIDA and VENZKE (1936) indicated that out of 36 cows, 23 had CL on the right ovary and 13 on the left. CLARK (1936) and CASIDA et al. (1948) collected ovaries from sexually immature heifers at a slaughter house, and found that 73.5% had dominant follicles and 60.2% had CL on the right ovary. In pregnant cows, 66.1% had CL on the right ovary. Because the right ovary is functionally more active than the left ovary, there are more pregnancies on the right side. BUCH et al. (1955) found a significant correlation between the time interval from calving to the oestrus and involution of the uterus in dairy cows.

According to HAMMOND (1927) most follicles developed on the ovary contralateral to the side of the previous ovulation. SAIDUDDIN et al. (1967) proved that in most cases the first postpartal ovulation took place on the ovary contralateral to the previously gravid horn. But, after the delivery, in only 51% of cases was the first ovulation on the right ovary. This percentage is smaller than in most other authors, probably because they registered not only the first, but also ovulations that occurred later after the delivery. FOOTE and PETERSON (1968) established in both beef and dairy cows, that folliculogenesis and ovulation are more frequent on the ovary contralateral to the side of previous pregnancy, and this phenomenon is even more pronounced in dairy cows. With the extension of the interval from calving to the occurrence of cyclic activity, this relation decreases. On the other hand, the average duration of this interval did not differ depending on the side of pregnancy. Cows that had the first ovulation after calving contralateral to the side of the previous gestation had better conception rates.

The aim of this study was to prove the bilateral functional asymmetry of the reproductive system in Holstein-Friesian cows, based on the side of the gravid horn, sides on which ovulation took place, as well as the results of conception in the horn ipsilateral and contralateral to the side of the previous pregnancy. Being able to determine the side of the reproductive system in which the next pregnancy should take place is important for biotechnological procedures, such as embryo transfer.

#### Materials and methods

*Animals*. This study was carried out in the period from 30<sup>th</sup> January 2007 to 20<sup>th</sup> October 2009 on four dairy farms, where 3008 cows were examined by rectal palpation and ultrasound examination, and 2300 of them were included in this study. All cows were of the Holstein-Friesian breed. Available data were used as a base for studying the effect of the side of pregnancy on the activity of the reproductive system and recent pregnancy.

Out of 2300 cows, 937 (40.74%) were in the first lactation, 641 (27.78%) in the second, 417 (18.13%) in the third and 305 (13.26%) in the fourth lactation, and in all of them it was the first manifested heat. In 2009 cows the occurrence of this oestrus was

spontaneous, and in 201 cows it was induced by the GPG (Ovsynch) method. Cows that had given birth to a single calf were included in this study, and it was registered on which side of the uterine horn the calf had been (right or left horn/ right or left pregnancy).

*Nutrition.* All cows were kept in intensive farming systems in similar conditions and fed with the identical type of food. This was food for cows in reproduction. They received 26.38 kg of dry matter in full meal of 47.99 kg, with 34.60 kg of fibre and 13.39 kg of concentrated food. The meal was composed of corn silage, lucerne silage, maize, grass silage, triticale, toasted soy, soy pellet, sunflower pellet, hay, straw, premix, sodium bicarbonate, calcium carbonate and magnesium oxide.

*Objects*. The four farms included in this research were in identical climate conditions, separated less than 20 km from each other. Cows were kept free indoors, and each building had a capacity of 230- 360 dairy cows. On three farms milking parlours were of the herringbone type and in the fourth farm of the rotary type.

Gynaecological examination. Cows scheduled for examination were directed from the milking parlours to the examination room, where they were examined within ten minutes. Sedatives were not used during the examination. Gynaecological examination and pregnancy diagnostics were conducted by the same expert, and artificial inseminations were performed by six veterinarians working on these farms.

The first examination. The first postpartal examination by rectal palpation and ultrasound examination, with control of ovarian structures and involution of the uterus was performed between the 25<sup>th</sup> and the 30<sup>th</sup> day postpartum. The horn that was in involution was recognised due to asymmetry in size and swelling. Cows with clinical complications and diseases from calving at this stage of first postpartal insemination were not included in this research.

The second examination. On the second examination, minimally 40 and maximally 100 days postpartum, the cows were selected for artificial insemination. Our intention was to register the cows in their first visible heat. Oestrus was detected by rectal palpation and ultrasound examination, and cows with follicles more than 12mm in diameter and of elastic to fluctuating consistency were selected for this study. Cows without detected dominant follicles were excluded from the research. Cows with mature follicles were artificially inseminated immediately after examination or within 8 hours afterwards, depending on the size and maturity of the dominant follicle.

The third examination. With the third examination, 15 to 20 hours after the artificial insemination (AI), ovulation was confirmed by rectal palpation and ultrasound examination. With the second examination, CL was detected in 2300 cows.

*Ultrasound examinations* were performed with devices "Scanner 100 Vet" (Pie Medical) with a linear probe (7,5 MHz, B-mode) and "Chison 600" (B-mode, linear probe 5,0-7,5 MHZ).

Artificial insemination. Cows were artificially inseminated with thawed semen deposited deep into the cranial part of the horn adjacent to the ovary with the ovulating follicle.

*Pregnancy diagnosis*. The side of the pregnancy as well as pregnancy diagnosis was performed by rectal palpation and ultrasound examination.

The fourth examination was performed 28 to 35 days after AI. The gravid side was established by palpation of the asymmetry of the pregnant uterine horn and ipsilateral ovaries containing CL. The anechogenic area in the gravid horn and CL on the adjacent ovary were confirmed by ultrasound examination.

*The fifth examination*. Between 60 and 80 days after AI, pregnancy was once more confirmed by rectal palpation.

Statistical analysis. Data were analysed with standard statistic methods used for processing of percentages and with the chi-square test (Sigma Plot 11.0). In the chi-square test, the value P=0.01 ( $\chi^2$ =6.63) was used as a limit value with one degree of freedom (d.f.). Results larger than this limit value were considered statistically significant.

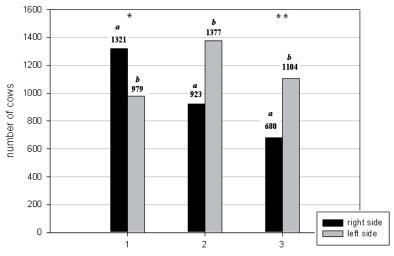
#### Results

From 2300 cows included in this research, in 1321 (57.43%) cows, the previous pregnancy was in the right horn and in 979 (42.57%) in the left horn. The difference between the side of pregnancy is statistically significant (P<0,01,  $\chi^2$ =50,852, d.f.=1) (Table 1).

Table 1. Distribution of gestation depending on the side of the reproductive system in previous and recent pregnancies

Side of the gravid horn	Previous N	Pregnancy %	Recent N	Pregnancy %
Left	979ª*	42.57	1104**	61.88
Right	1321b*	57.43	680b**	38.12
Total	2300		1784	

a,b rows with different superscript differ significantly (P<0.01); \*,\*\* columns with different superscript differ significantly (P<0.01)



side of reproductive system

Fig. 1. The side (left or right) of the reproductive system of cows with previous pregnancy (1.), postpartal ovulation (2.) and recent pregnancy (3.);  $^{a,b}$  columns with different superscript differ significantly (P<0.01);  $^*$ ,  $^{**}$  group of columns with different superscript differ significantly (P<0.01)

On the second postpartal examination, between 40 and 100 days postpartum, there were 1377 (59.86%) ovulations on the left ovary and 923 (40.14%) on the right ovary. This is also a statistically significant difference (P<0.01,  $\chi^2$ =89.6, d.f.=1). Therefore, the occurrence of ovulation is more significant on the ovary contralateral to the side of the previous pregnancy (Fig. 1).

Table 2. The ratio of cows that conceived to the total number of cows; the ratio of cows with recent pregnancy ipsi- and contralateral to the side of previous pregnancy to the total number of cows, and the number of cows that conceived with postpartal ovulation on the same side

Cows	N	Total number of cows (%)	Total number of cows that have conceived (%)	Postpartal ovulation on the same side (%)
Conceived (total)	1784	77.56		
Contralateral ovulation (L)	1104	48	61.88	80.17
Ipsilateral ovulation (D)	680	29.56	38.12	73.67

After AI, performed between 40 and 100 days postpartum, 1784 cows out of 2300 (77.56%) conceived. In 1104 out of 1784 cows (61.88%) pregnancy was on the left side. That is 48% of the total number of cows in the research, 61.88% of the cows that conceived and 80.17% of the cows that ovulated on the ovary contralateral to the side of the previous gestation. In only 680 (38.12%) cows was the pregnancy on the right side, ipsilateral to the previous side of pregnancy (Table 2).

The distribution of embryos in the left or right uterine horn in dairy cows is statistically significant (P<0.01,  $\chi^2$ =100.76, d.f.=1). There is also a statistically significant difference in the frequencies of the sides of pregnancy between the previous and recent gestation (P<0.01,  $\chi^2$ =272.94, d.f.=1) (Fig. 1).

#### **Discussion**

The significantly higher occurrence of right side pregnancies is in compliance with data from many authors (listed in MORROW et al., 1968; PIERSON and GINTHER, 1987; LOPEZ-GATIUS and CAMON-URGEL, 1990). They have proved that a more active right side of the reproductive system is a physiological phenomenon in normally cycling cows, although there are authors whose studies disagree with this theory (CALLESEN et al., 1986; PALLARS et al., 1986). The reason for this difference in activity is thought to result from the fact that the right horn is bigger than the left one (PERKINS et al., 1954). Increased activity can have an effect on sperm transport, and subsequently on conception rates, as cited by LARSSON and LARSSON (1985 and 1986), who indicate that there was more sperm left in the left than in the right horn after AI of cows and heifers with single ovulations. Transuterine embryo migrations are sporadic in cows, except in cases of multiple ovulations, and, unlike in sheep, they do not affect ovarian activity in cows (SCANLON, 1972).

MORROW et al. (1968) confirmed that the right ovary was more active than the left one, but this only affects ovarian activity in the following ovulation. Corpus luteum of the following pregnancy was on the ovary contralateral to the side of the previous gestation. According to them, during the first 60 days postpartum, 139 (55.8%) of a total of 256 ovulations were on the right ovary. This frequency increased with the duration of the postpartal period from 47.8% in the 1st to 20th days to 63.4% in 40th to 60th days postpartum. Also, the occurrence of CL on the left ovary decreased from 52.2% in the 1st to 20th days to 36.6% in the 41st to 60th days postpartum. This indicates that the right ovary only becomes more active compared to the left one in the later part of the postpartal period. In the first 20 days postpartum there was more CL on the ovary contralateral to the side of previous gestation (P<0.005). Clinical research done by STRODHOFF (1922) indicates that ovulations ipsilateral to the side of previous ovulations are not very common. The reason for this is that CL directly inhibits folliculogenesis. In favour of this finding is the

fact that during normal oestrous cycle there are more middle-sized follicles on the ovary where there has not previously been any CL (MATTON et al., 1981).

SAIDUDDIN et al. (1967) also proved that the first postpartal ovulation occurs more frequently on the ovary contralateral to the side of the previous pregnancy. They studied the possible effect of CL on the post gravid horn on the side of the first postpartum ovulation in 136 cows of Holstein breed, in 206 inter-calving intervals, and established that the right ovary had stronger functional activity compared to the left one, because there were 60-64% of right side ovulations. According to the same authors, the postgravid horn blocks folliculogenesis on the adjacent ovary, because its sensibility is weakened. Possibly the lower percentage of first postpartum right-sided ovulations is evidence that even later occurring ovulations have been taken into account, indicating that the duration of the postpartum period is an important factor of ovarian activity. Similarly, FOOTE and PETERSON (1968) suggest that the postgravid horn has a direct unilateral inhibiting effect on ovarian activity. GUILBAULT et al. (1983 and 1987) proved that suppression with prostaglandine F2a in the postpartal period has no effect on the involution of uterus, but reduces the activity of the ovary contralateral to the one with CL in the previous pregnancy. MARION and GIER (1968) found that from 11 to 15 days postpartum in 92% of cases ovulation took place on the side opposite to the side of the previous pregnancy. Together with the inhibiting and regressing effect of CL, the possible reason is the embryotoxic effect of PGF2 $\alpha$ . In postpartal cows, the secretion of PGF2 $\alpha$  is continuous (HU et al., 1990; COOPER et al., 1991), and as CL is responsible for the secretion of luteal oxytocin (FIELDS and FIELDS, 1986; FUCHS, 1987; FLINT et al., 1990), this results in increased secretion of maternal PGF2a, with the consequent inhibition of activity and lower conception rates.

LOPEZ-GATIUS (1997) studied the relationship between the activity of the right and the left sides of the reproductive organs regarding sperm transport and the side of the previous pregnancy. He established higher right side activity that remained unchanged no matter on which side the previous pregnancy had been. In 55.8% of cows the first pregnancy was on the right side, and the first postpartal ovulation was also on the right side in 56.5% of cases. The same author (1996) studied the effect of the previous pregnancy on sperm transport and conception rates after deep intracornual AI in the first lactation. 52% of the cows ovulated on the ovary contralateral to the side of the previous pregnancy. Insemination into the previously non gravid horn resulted in higher conception rates. This indicates a negative effect on the semen. So, the side of sperm deposition (into the ipsilateral or contralateral horn regarding the side of ovulatory follicle) and the side of the previous pregnancy affect the conception rate (LOPEZ-GATIUS and CAMON-URGEL, 1988; LOPEZ-GATIUS, 1996).

In our research we found a significantly higher frequency of left side postpartal ovulations, i.e. in 59.86% cases it was on the side opposite to the previously gravid horn. Out of 2300 cows, 1784 (77.56%) conceived. 1104 of those cows conceived contralateral to the side of the previous pregnancy, i.e. 80.17% of cows ovulated on the side opposite to the previous pregnancy. There is an obvious significant difference in the frequency of embryo distribution regarding the side of the reproductive system. So, we have confirmed the existence of bilateral asymmetry in the activity of the reproductive organs, which is primarily the result of physiological differences in the tubular part of the system, but also of uneven ovarian function, as shown by LOPEZ- GATIUS (1997).

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#### SAŽETAK

Cilj istraživanja bio je usporediti odnos poslijeporođajne aktivnosti lijeve i desne strane reprodukcijskih organa na posljedičnu plodnost, uvjetovano stranom prethodne gestacije. U pokusu su korištene isključivo krave s pojedinačnim ovulacijama. Osjemenjivane su duboko intrakornualno, ipsilateralno u odnosu na stranu preovulatornoga folikula. Učinci su istraživani u 2300 frizijskih krava, od kojih je 937 (40,74%) bilo u prvoj laktaciji, 641 (27,87%) u drugoj, 417 (18,13%) u trećoj i 305 (13,26%) u četvrtoj laktaciji, od čega je u 2099 (91,26%) nastup prvoga izraženoga estrusa bio spontan, a u 201 krave (8,74%) estrus je bio induciran GPG (Ovsynch) metodom. Uspoređivan je odnos strana prethodne gestacije kao i rezultat koncepcije obzirom na stranu gdje se nalazio ovulirani jajnik. Zabilježena je značajno pojačana aktivnost desne strane reprodukcijskoga sustava u prethodnoj gestaciji. Nakon involucije maternice, zabilježena je pojačana aktivnost kontralateralno od prethodne gestacije. Odnos strana ovuliranih jajnika statistički je značajan, ukazujući da je pojavnost ovulacije učestalija na jajniku nasuprotnom postgravidnom rogu. Rezultati upućuju na to da je bilateralna asimetrija reproduktivnoga sustava u tjecala na koncepciju krava i potvrđuju asimetriju funkcije spolnih organa, kako zbog neujednačene jajničke aktivnosti, vjerojatno još i više zbog fiziološke razlike cjevastoga dijela organa, uvjetovano stranom prethodne steonosti i involucijom materničnoga postgravidnoga roga.

Ključne riječi: mliječne krave, obostrana asimetričnost, strana steonosti, strana ovulacije