

Superovulatory response in relation to the size and side of ovary location in high yielding dairy cows on the first day of treatment protocol

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ABSTRACT

The aim of this research was to determine the relation between the size of ovaries, their topographic position, ovarian structures (follicles (F) and corpora lutea (CL)) before superovulatory treatment and the superovulatory response and embryo yield. Holsteinized dairy cows (32) were treated for superovulation with Pluset® (Laboratorios Calier, S.A., Barselona, Spain). On Day 1 of the superovulatory treatment and during the procedure to recover the embryos, we examined the ovaries of donors with an ultrasound scanner. The ovaries were divided into 3 groups according to their size. Group 1 ovaries (<668.0 mm²) were more than 25 % smaller and Group 3 ovaries (>1112.0 mm²) were 25 % larger than Group 2 ovaries, the area of which varied from 668.0 to 1112.0 mm² (mean, 890.0 mm² ± 25 %). After superovulation, the mean area of the right side ovaries (RSO) (3147.61 ± 106.6 mm²) was by 27.3 % (P<0.05) larger than the mean area of the left side ovaries (LSO). Irrespective of location, the ovaries after superovulation increased 3.2 times; however, the increase in the RSO, except for Group 3, was 14 % more intense (P>0.05) compared with the LSO. The most intense response to the superovulatory treatment by ovary size was observed in Group 1 ovaries: the RSO increased by 5.5 times and the LSO by 4.8 times, but according to the absolute measure, the greatest increase was observed in Group 3, where ovaries on both sides increased 2.4 times. According to the number of CL, Group 1 RSO donors showed a 2.3 times (P<0.05) weaker response to the superovulatory treatment in comparison with Group 3 RSO donors. The number of embryos recovered (9.8 ± 2.2) was 3.6 times (P<0.05) higher among the donors with Group 3 RSO before superovulation, compared with the donors' Group 1 RSO (2.7 ± 0.5). The number of transferable embryos was 3.2 times (P<0.05) higher in cows with Group 3 (6.3 ± 0.6) RSO in comparison with the donors' Group 1 (2.0 ± 0.3) RSO. Corpora lutea in the RSO demonstrated a significant impact of 85 % (P<0.05) on the yield of embryos, compared with only 30 % (P<0.05) for the LSO.

Key words: donors, cows, superovulatory treatment, superovulatory response, embryo yield

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Introduction

Embryo transfer is usually performed in dry dairy cows. However, such cows are subjected to stricter selection requirements, as milk production is one of the health risk factors (MAILLO et al., 2012). The response of dairy cows to the superovulatory treatment is hard to predict. Ovarian response to superovulatory treatment depends on a series of factors: the milk production, negative energy balance, general health condition, and individual characteristics of the animals (CABRERA et al., 2013; KUBOVIČOVÁ et al., 2013; TAMADON et al., 2011). According to SEIDEL et al. (2003), 27 % of all healthy donors produce from 0 to 1 embryo, 54 % of donors from 2 to 10 embryos, and only 20 % of donors produce 11 or more embryos. Similar data were presented by SCHATTEN and CONSTANTINESCU (2007), who stated that not a single embryo was flushed from 24 % of cow-donors and as much as 70 % of flushed embryos were obtained from 30 % of donors. No unanimous opinion about the selection criteria for donors has been achieved between researchers. Nevertheless, a certain correlation between ovarian structures and the response to the superovulatory treatment has been noted (GRIZELJ et al., 2013). Ultrasound examinations of animals have become increasingly used in practice. Thus, it should be analysed how cows - potential donors of embryos - are to be assessed and selected for embryo production. The method described in this study may be used for this purpose.

The size of ovaries is closely related to the wave-like manner in normal cycling cattle (PENG et al., 2011). The size of an ovary is determined by functional formations, i.e. follicles (F) and corpora lutea (CL) (CUSHMAN et al., 2010). The size of the CL correlates with the concentration of progesterone (KAYACIK et al., 2005), and cows with increased concentrations of progesterone demonstrate a more active and effective response at the beginning of a superovulation scheme compared with cows with reduced concentrations of progesterone (KUDLAČ et al., 1991). The ovarian function in cows depends on the presence of primordial follicles (SILVA-SANTOS et al., 2013). A large number of such follicles in the contralateral ovary correlates with the number of CL after induction of polyovulation (CUSHMAN et al., 1999). A cow is considered to be more suitable as a donor cow and produce more embryos when there are no large F at the beginning of induction of superovulation (HELEIL and EL DEEB, 2010). A certain morphological and functional asymmetry of ovaries has been noted. Past gestations have demonstrated that the right ovary is more active than the left one by 14 % (GEREŠ et al., 2011).

The aim of this work was to assess by ovarian ultrasonography the relationship between the size of ovaries, their left/right side position, ovarian structures (F and CL), the superovulatory response, and embryo yield.

Materials and methods

For the purposes of this study, we selected 32 clinically healthy holsteinized Lithuanian Black and White cows, held in a loose-housing system and which had calved 75-90 days before; their productivity during the previous lactation was 9,500-10,500 kg.

The donors' daily ration included corn silos (24 kg), haylage (11 kg), barley straw (0.8 kg), molasses (0.5 kg), and composite feed (8.74 kg) composed of dried sugar-beet slices (20.59 %), soy bean meal (17.16 %, of which 49 % protein content), triticale (34.32 %), rape cake (22.88 %), Bergophor CaProVit H mineral (2.29 %), fodder limescale (0.8 %), salt (NaCl, 0.34 %), monocalcium phosphate (0.69 %), and sodium bicarbonate (0.93 %).

For the study, only the cows with CL larger than 10 mm and no follicle larger than 10 mm in the right or left ovary were selected before starting the superovulatory treatment.

The donors were treated for superovulation with Pluset® (follicle stimulating hormone (FSH) and luteinizing hormone (LH) at the ratio 1:1; Laboratorios Calier, S.A., Barcelona, Spain) at any time of the oestrus cycle. As for the solution, 1 mL of Pluset® contained 35 IU FSH and 35 IU LH and the total dose per donor was 700 IU.

The superovulatory treatment was induced according to the following scheme (Table 1) (LAFRI et al., 2002; SEIDEL et al., 2003).

Table 1. Superovulatory treatment of donors

No	Days of superovulation		Procedures performed
1.	Day 1		Introduction of intravaginal implant CIDR containing 1.38 g of progesterone
2.	Day 6	Morning	I/m injection of 4 mL of Pluset 140 IU
		Evening	I/m injection of 4 mL of Pluset 140 IU
3.	Day 7	Morning	I/m injection of 3 mL of Pluset 105 IU
		Evening	I/m injection of 3 mL of Pluset 105 IU
4.	Day 8	Morning	I/m injection of: - 2 mL of Cloprostenol - 2 mL of Pluset 70 IU
		Evening	I/m injection of: - 2 mL of Cloprostenol - 2 mL of Pluset 70 IU
5.	Day 9	Morning	- removal of intravaginal implant CIDR - i/m injection of 1 mL of Pluset 35 IU
		Evening	I/m injection of 1 mL of Pluset 35 IU
6.	Days 10-11		Artificial insemination
7.	Day 18		Embryo recovery

The insemination was performed on Days 10-11 according to the signs of the oestrus (twice at an interval of 12 hours). Embryos were collected after 7 days, using a nonsurgical embryo recovery method with a Rusch catheter. The embryos were flushed with BoviFlush medium with BSA and antibiotics, manufactured by Minitube, Germany. For each flushing we used 1000 mL of medium per donor.

On Day 1 of superovulatory treatment, before inserting the implant Pride of Progesterone (CIDR[®], Pfizer, Inc.), and on Day 18 when the embryos were recovered, the length and width of the ovaries and the number of ovarian structures (F and CL) were examined using Digital Diagnostic Ultrasound Devices (HG 9300, Caresono Technology Co., Ltd) with a 7 MHz rectal transducer. On Day 18, the ovaries were examined by ultrasonography and the efficiency of superovulation was determined according to the number of CL and the ovary size. The sizes of the ovaries were calculated by multiplying the widths and lengths, and the ovaries were divided into 3 groups according to the ovary area. Group 1 (<668.0 mm²) ovaries were 25 % smaller and Group 3 ovaries (>1112.0 mm²) were 25 % larger than Group 2 ovaries (890.0 mm² ± 25 %), with ovary areas varying from 668.0 to 1112.0 mm² (Table 2):

Table 2. Groups of ovaries based on their area (the product of width and length) before superovulatory treatment

Ovaries groups	Ovaries size before superovulatory treatment (mm ²)
No. 1	<668.0
No. 2	668.0 - 1112.0
No. 3	>1112.0

RSO- right ovary size; F-follicle; CL-corpora luteum The data were statistically processed using the SPSS statistical package (IBM SPSS for Windows 20.0 SPSS Inc., Chicago, IL, USA, 2011). For the statistical analysis of the data, descriptive statistics and the multifactor analysis ANOVA test were used. The data were considered statistically significant when $P < 0.001$; $P < 0.05$.

Results

The mean ovary area (990.21 ± 57.3 mm²) on the right side in the donors, before starting superovulation, was 26.6 % ($P < 0.05$) larger than the mean ovary area on the left side (Table 3).

Before superovulation, the RSO were assessed in 62.5 % of Group 2 and LSO in 50 % of Group 1.

Table 3. Ovary area (mm²) depending on the side of location before and after superovulatory treatment

Ovaries groups	Before superovulatory treatment		After superovulatory treatment	
	Left ovary	Right ovary	Left ovary	Right ovary
No.1	428.5 ± 23.3	441.33 ± 84.6	2047.67 ± 112.4	2410.67 ± 18.4
No.2	850.75 ± 17.45	883.0 ± 151.78	2334.0 ± 53.7	2661.50 ± 123.2
No.3	1244.5 ± 125.92	1825.0 ± 70.9	3036.0 ± 64.2	4370.67 ± 205.7
Average:	782.29 ± 37.7 ^a	990.21 ± 57.3 ^b	2472.6 ± 50.5 ^c	3147.61 ± 106.6 ^d
Average in total:	886.25 ± 487.9		2810.08 ± 133.7	

a,b, c,d, rows with different superscript differ significantly (P<0.05)

After superovulation, the mean size of the RSO (3147.61 ± 106.6 mm²) was 27.3 % (P<0.05) larger compared with the LSO. Irrespective of location, after superovulation, the ovaries increased on the average 3.2 times; however, the increase in the RSO, except for Group 3, was 14 % (P>0.05) more intense compared with the LSO. It was determined that in Group 1 the RSO increased by 5.5 times and the LSO by 4.8 times. In Group 2 ovaries on both sides increased on average by 2.7-3.0 times. The smallest increase was observed in Group 3 ovaries on both sides, of only about 2.4 times. According to the absolute measure, the greatest increase after superovulation was observed in Group 3 RSO.

The results demonstrated a moderate but statistically significant correlation between the sizes of the RSO and LSO (Table 4). In fact, if the ovaries are bigger on one side, they will also be bigger on the other side. The ovary size is determined by the expression of ovarian structures. No statistically significant or reliable relationship between the ovary size and the number of CL was established. The size of the RSO showed (r = 0.865, P<0.005) a 2.1 times (P<0.05) stronger correlation with the number of F in comparison with the left ovary (r = 0.42, P<0.05). The number of F appeared to have an essential impact on the size of the ovaries.

Table 4. Statistical relation between ovary size and ovarian structures before superovulatory treatment

Structures of ovaries	Pearson correlation	Left ovary		Right ovary	
		Size	F	Size	F
Right ovary size	r	0.549*	0.287	1	0.865**
Right ovary follicles	r	0.636*	0.625*	0.865**	1
Right ovary corpora lutea	r	-0.236	-0.866	0.002	-0.322
Left ovary follicles	r	0.42*	1	0.549*	0.636*
Left ovary corpora lutea	r	0.631	0.24	0.287	0.625

rows with ** coefficient of correlation significance (P<0.005); rows with * coefficient of correlation significance (P<0.05); F-follicle

The size of the RSO and LSO before superovulation correlated statistically reliably with the size (right $r = 0.648$, $P < 0.05$ and left $r = 0.708$, $P < 0.001$), and with the CL (right $r = 0.758$, $P < 0.05$ and left $r = 0.723$, $P < 0.05$) of RSO after superovulation. The size of the LSO before superovulation demonstrated a statistically reliable ($P < 0.05$) correlation with the number of F of LSO after superovulation.

Table 5. Statistical relation between sizes of ovaries before superovulatory treatment and ovarian structures after superovulatory treatment

Before superovulatory treatment	Pearson correlation	After superovulatory treatment					
		Left ovary			Right ovary		
		Size	F	CL	Size	F	CL
Right ovary size	r	0.15	0.58	0.055	0.648*	0.30	0.758*
Left ovary size	r	0.430	0.686*	0.066	0.708**	0.597	0.723*

row with ** coefficient of correlation significancy ($P < 0.005$); row with * coefficient of correlation significancy ($P < 0.05$); F-follicle; CL-corporus luteum

According to the number of CL, the most effective superovulation was observed in Group 3 ovaries. The number of CL in the RSO and LSO after the superovulatory treatment depended on the group size. In Group 3 RSO, the number of CL (9.0 ± 3.0) was 2.3 ($P < 0.05$) and 1.6 times greater in comparison with Group 1 and Group 2, respectively. The number of CL in the LSO was similar (mean 3.5 CL) in the first and second group, but in Group 3 the number (5.71 ± 0.8) of CL was 1.6 times greater compared with Group 1 and Group 2. The lowest number of F was observed in Group 2 in ovaries on both sides.

Table 6. Quantitative results of superovulation in relation to groups of ovaries before superovulatory treatment

Ovaries groups	Left ovary		Right ovary	
	F number	CL number	F number	CL number
No.1	3.50 ± 0.5	3.52 ± 0.1	6.30 ± 0.1	4.00 ± 0.1^c
No.2	3.34 ± 0.54^a	3.57 ± 0.88	3.29 ± 0.94	5.67 ± 1.2
No.3	4.40 ± 0.1^b	5.71 ± 0.8	4.12 ± 2.02	9.00 ± 3.0^d

a:b, c:d, columns with different superscript differ significance ($P < 0.05$); F-follicle; CL-corporus luteum

It was established that the greatest statistical impact (significance 85 %, $P < 0.05$) on the yield of embryos was demonstrated by CL found in the RSO after superovulation, whereas CL on the LSO demonstrated an almost 3 times smaller impact (significance 30 %, $P < 0.05$). Thus, a statistically reliable and significant impact (significance 38 %, $P < 0.05$) on the yield of embryos was demonstrated by CL in the RSO before superovulatory treatment (Table 7).

Table 7. Ovarian structures statistical significance to embryos yield

Factor	Significance (%)	P
Right ovary corpus luteum after superovulation	85	0.03
Left ovary corpus luteum after superovulation	30	0.04
Right ovary corpus luteum before superovulation	38	0.01
Left ovary corpus luteum before superovulation	0	0
Right ovary follicle before superovulation	21	0.02
Left ovary follicle before superovulation	23	0.17

Multifactorial analysis. Ovarian structures are dependent variables and embryo yield - fixed factor.

As shown in Table 8, Group 3 ovaries had more F before superovulation. It was noted that the size of the ovary before superovulation had a direct effect on the yield of embryos and the number of transferable embryos. The largest number of embryos was obtained from Group 3 donors. The 3.6 times ($P < 0.05$) larger number of embryos was obtained from Group 3 RSO donors (9.8 ± 2.2) compared with Group 1 RSO donors (2.7 ± 0.5) and 1.9 times more embryos compared with Group 2 RSO donors (5.2 ± 1.2). On the average, Group 3 RSO donors yielded 6.3 transferable embryos, which were 3.2 times ($P < 0.05$) more compared with Group 1 RSO and 1.6 times more compared with Group 2 RSO donors.

Table 8. Differences between right ovary size and mean number of corpora lutea, mean number of follicles before superovulation and mean number of recovered embryos

Groups by RSO	Mean number of CL (>10 mm)/donor (unit)	Mean number of F (<10 mm)/donor (unit)	Mean number of embryos recovered per donor (unit)	Mean number of transferable embryos recovered per donor (unit)
No.1	1.0 ± 0.1	1.6 ± 0.6	2.7 ± 0.5^a	2.0 ± 0.3^d
No.2	1.6 ± 0.3	2.4 ± 0.8	5.2 ± 1.2^b	3.9 ± 0.4^c
No.3	1.25 ± 0.3	3.3 ± 1.1	9.8 ± 2.2^c	6.3 ± 0.6^f

a,c, d,f, columns with different superscript differ significance ($P < 0.05$); a:b, d:e, b:c, e:f, columns with different superscript differ not significantly ($P > 0.05$)

Discussion

A major limitation to the development of embryo production in cattle is the strong between-animal variability in ovulatory response to FSH-induced superovulation (RICO et al., 2009).

The superstimulatory response can be predicted by an ultrasound-based test of the ovaries (SINGH et al., 2004). Functional asymmetry of ovaries has been observed (SCHNEEBELI and DÖBELI, 1991).

ALVAREZ et al. (2005) noticed the asymmetry of ovarian function in their study on the impact of inbreeding on the ovary response to superovulation. A cow's embryo more often develops in the right uterine horn (GIRALDO et al., 2010). The asymmetry of cow reproduction physiology can be illustrated by the fact that embryos recovered from the right uterine horn are mostly male (HYLAN et al., 2009). According to the data of other authors, bilateral genital tract asymmetry in cows affects progesterone, proteins, and hexoses, without altering pregnancy rates (TRIGAL et al., 2013). We estimated that the right ovary was not only larger in its linear size but also that it reacts more actively to the superovulatory treatment with gonadotropins. Group 1 RSO were less frequently determined than Group 1 LSO. According to the size of ovaries after superovulation, Group 1 RSO donors demonstrated a 1.8 times weaker response to the superovulatory treatment compared with Group 3 RSO donors. According to the number of CL, Group 1 RSO donors showed a 2.3 times ($P < 0.05$) weaker response to the superovulatory treatment in comparison with Group 3 RSO donors. Group 1 RSO (smaller than 668.0 mm²) were found in one third of all the selected donors. Small Group 1 ovaries on both sides signal a reasonable risk that the donor would demonstrate poor response to the superovulatory treatment, and yield a small number of embryos. The functional asymmetry needs to be taken into account for assessment of ovarian condition.

During the cycle of reproduction, follicles are constantly found in the ovaries (SIROIS and FORTUNE, 1988). The number of well expressed formations - CL and F - in an ovary depends on the growth of follicles. The population of ovarian F is a permanent formation present during the entire cycle of reproduction. Research has shown that the size of an ovary mostly depends on the number and size of F (SIROIS and FORTUNE, 1988). CUSHMAN et al. (1999) claimed that the ovarian response to the superovulatory treatment was dependent on the population of small follicles in the ovary.

It has been noted that the smaller the CL, the larger the F (LÜTTGENAU et al., 2011). It has been previously described that the response to gonadotropin superstimulation depends on the size of F: under the effect of chorionic gonadotropin, functional F (larger than 17.0 mm in diameter) mature and ovulate, whereas atretic F become luteinized (MONNIAUX et al., 1983). Meanwhile, KOHRAM and POORHAMDOLLAH (2012) found that ovaries with larger F (9.8 and 10.1 mm), found 4 days before superovulation, demonstrated a more active response to the superovulatory treatment and produced a larger number of good quality embryos.

The statistical significance of CL determined before superovulation for the yield of embryos may be explained as the significance of a physiological marker showing undisturbed steroid metabolism in cows. There is a high correlation between the diameter of CL and the plasma progesterone concentration (RIBADU et al., 1994). CL is also a signal showing that there is no dominating F in the ovaries of a healthy cow.

Development of F is also affected by high productivity of cows and negative energy balance (LUCY et al., 1992). According to MAPLETOFT et al. (2002), the response to the superovulatory treatment depends on the growth of F, which may be controlled with steroids.

Since it is known that progestogens inhibit the development of dominating F and stimulate a new wave of follicular growth, which contributes to the response to gonadotropin superstimulation (BO et al., 1995), only donors that had active CL larger than 10 mm and smaller than 10 mm F in their ovaries were selected as the donors for this study.

The size of the RSO and its ovarian formations before the superovulatory treatment are of special importance for prediction of the efficiency of the superovulatory treatment and the yield of embryos. For donors with average ovary areas (multiplied length and width) larger than 1112.0 mm², well-developed CL in the RSO (at least 10.0 mm in size), and one F or several F (smaller than 10.0 mm) before superovulation, the chances for successful superovulatory response are 1.8 times greater by size of the ovaries and 2.3 times ($P<0.05$) greater by number of CL after the superovulatory treatment, compared with the RSO smaller than 668.0 mm².

The number embryos recovered from Group 3 RSO donors was 3.6 times ($P<0.05$) larger compared with Group 1 RSO donors, and 1.9 times larger compared with Group 2 RSO donors. The donors with Group 3 RSO provided 3.2 times ($P<0.05$) more transferable embryos compared with Group 1 RSO donors.

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PALUBINSKAS, G., V. ŽILAITIS, V. JUOZAITIENĖ, L. LAUČIENĖ, V. JUODŽENTIS, A. GAVELIS, A. SEDEREVIČIUS: Superovulacijski odgovor prvog dana liječenja i njegova povezanost s veličinom i položajem jajnika kod visokoproduktivnih mliječnih krava. *Vet. arhiv* 86, 65-76, 2016.

SAŽETAK

Cilj ovog istraživanja bio je utvrditi povezanost veličine, položaja i građe (folikuli F i žuto tijelo CL) jajnika prije superovulacijskog postupka te nakon superovulacijskog odgovora i dobivenih embrija. Kod 32 mliječne krave u tipu holštajna, superovulacija je provedena primjenom Pluset® (Laboratorios Calier, S.A., Barcelona, Španjolska). Tijekom 1. dana superovulacijskog postupka i oporavka embrija, jajnici davateljica bili su pretraženi ultrazvukom. S obzirom na veličinu jajnici su bili podijeljeni u tri skupine. Skupinu 2 činili su jajnici čija je veličina varirala od 668,0 do 1112,0 mm² (srednja vrijednost, 890,0 mm² ± 25 %), skupinu 1 (<668,0 mm²) činili su jajnici s više od 25 % manjom veličinom od skupine 2, a skupinu 3 jajnici (>1112,0

mm²) više od 25 % veći od jajnika skupine 2. Nakon postupka superovulacije, srednja veličina ($3147,61 \pm 106,6$ mm²) desnih jajnika (DJ) bila je 27,3 % ($P < 0,05$) veća nego srednja veličina lijevih jajnika (LJ). Neovisno od položaja, jajnici su nakon superovulacije bili povećani 3,2 puta, međutim povećanje je kod DJ, osim za skupinu 3, bilo 14 % izraženije ($P > 0,05$) u usporedbi sa LJ. Najintenzivniji superovulacijski odgovor s obzirom na veličinu jajnika opažen je u skupini 1: kod DJ povećanje je bilo 5,5, a kod LJ 4,8 puta, ali s obzirom na apsolutne mjere, najveći porast opažen je u skupini 3 u kojoj su jajnici s obje strane povećani 2,4 puta. S obzirom na broj CL, skupina 1 DJ davateljica pokazala je 2,3 puta ($P < 0,05$) slabiji superovulacijski odgovor u usporedbi sa skupinom 3 DJ davateljica. Broj dobivenih embrija ($9,8 \pm 2,2$) bio je 3,6 puta ($P < 0,05$) veći među davateljicama 3 DJ skupine prije superovulacije u usporedbi sa davateljicama skupine 1 DJ ($2,7 \pm 0,5$). Kod krava skupine 3 DJ, broj prenosivih embrija ($6,3 \pm 0,6$) bio je 3,2 puta ($P < 0,05$) veći u usporedbi s kravama davateljicama skupine 1 DJ ($2,0 \pm 0,3$). Žuta tijela u DJ su pokazala signifikantni utjecaj od 85 % ($P < 0,05$) na dobivanje embrija u usporedbi sa samo 30 % ($P < 0,05$) u LJ.

Ključne riječi: davateljice, krave, superovulacijski postupak, superovulacijski odgovor, broj embrija
