Ovarian activity and embryo yield in relation to the postpartum period in superovulated dairy cows

Luděk Stádník[1], Jiří Bezdíček[2], Alexander Makarevich[4], Elena Kubovičová[4], František Louda[3], Ivana Fellnerová[2], Zdeňka Hegedüšová[5], Radek Holásek[5]

[1]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Animal Husbandry, Prague, Czech Republic
[2]Palacký University Olomouc, Faculty of Science, Department of Zoology and Laboratory of Ornithology, Olomouc, Czech Republic
[3]Research Institute for Cattle Breeding Rapotin, Ltd., Department of Feed, Nutrition, Breeding and Reproduction, Vikyrovice, Czech Republic
[4]Research Institute for Animal Production Nitra, National Agricultural and Food Centre (NAFC), Lužianky-near-Nitra, Slovak Republic
[5]Taura ET Ltd., Litomyšl, Czech Republic

Received March 15, 2016
Accepted February 17, 2017

Abstract

The aim of this study was to evaluate superovulation response in cows at various postpartum periods (early postpartum period up to 3.5 months; middle postpartum period 3.7–7 months; later postpartum period above 7.5 months after calving). The data included observation of 55 Holstein cows superovulated at one farm in the Czech Republic during the years 2010 and 2013. Reproduction traits (dependent variable) were represented as number of the corpora lutea, number of transferable embryos, morulae, blastocysts, total number of embryos and embryo recovery. For statistical evaluation we used the PROC GLM of SAS® with fixed effect - breeding value of milk production. The study results show significant differences ($P < 0.05$–0.01) in the three postpartum periods (early, middle, and later postpartum periods) and the number of corpora lutea (4.6; 7.4; 10.8), number of total embryos (3.2; 2.9; 6.5) and transferable embryos (1.8; 1.7; 4.4). Effective timing of embryo transfer in the later postpartum period resulted in greater ovarian activity and embryo yield compared to early lactation periods.

Superovulation, corpus luteum, transferable embryos

Milk production and reproduction are two important physiological traits that have consistently demonstrated a negative correlation in cows (Stádník and Louda 1999) due to the physiological adaptation of cows to excessive milk production in recent years (Lucy 2001). Both traits are related to individual genetics (Bezdíček et al. 2007). However, deteriorating reproduction in recent years is also associated with the level of inbreeding (Bezdíček et al. 2008; Bezdíček et al. 2014) and the quality of the environment has an important impact, too. Good reproduction in dairy cows is associated with a period of sharp increase in milk production in early lactation, connected to increased demands on the metabolism of the whole organism (Stádník et al. 2015) in maintaining the physiological condition of the cow (Stádník et al. 2002) and allowing utilization of energy resources to provide the necessary endocrine indicators (Royal et al. 2000; Beran et al. 2013).

The prenatal period, birth, and the start of the lactation period are significantly associated with large energy demands on the body. The results of a number of studies have shown that in early lactation, animals are under greater physiological stress than in later postpartal periods. Bauman and Currie (1980) reported that cows use aproximately 50 kg of pure lipid of body energy reserves during the first 10 weeks of lactation. Villa-Godoy et al. (1988) pointed out the fact that the caloric deficit can be one reason for infertility in lactating

Address for correspondence:
Luděk Stádník
Czech University of Life Sciences Prague
Kamýcká 129, 165 21, Praha 6 – Suchdol, Czech Republic
Phone: +420 224 383 057
E-mail: stadnik@af.czu.cz
http://actavet.vfu.cz/

dairy cows. Butler (2000) added that a negative energy balance can delay the time of first ovulation through inhibition of the luteal hormone (LH) and low concentrations of blood glucose, insulin, and insulin-like growth factor-I (IGF-I) followed by inhibition of oestrogen production by dominant follicles.

Loker et al. (2009) found that cows with ≤ 180 days open tended to have low yields in the last part of lactation. Cows with longer days open had a proportionally higher milk yield throughout lactation, suggesting a possible confounding effect of prolonged days open.

Maillo et al. (2012) studied the direct effect of lactation (lactating and dry cows) on embryo development in Holstein cows, reporting that 26.3% of total embryos developed to the blastocyst stage in the lactating cows compared to 39.6% in the nonlactating groups. Leroy et al. (2005) who compared embryo quality in lactating Holstein cows, non-lactating Holstein heifers, and Belgian Blue beef cows concluded that lactating cows yielded embryos with a significantly reduced quality (only 13.1% embryos categorized as excellent vs. 62.5% in non-lactating heifers, and 55.0% in Belgian Blue cows). A lower percentage of blastocyst stage (4%) was also found in these dairy cows compared to 23.2% in heifers and 17.3% in Belgian Blues.

Sartori et al. (2002) found lower embryo quality in lactating cows compared to dry cows (3.1 vs 2.2 points; on a scale of 1 = excellent to 5 = degenerate), as well as in lactating cows compared to heifers (3.8 vs 2.2 points).

Rizos et al. (2010) also reported that the reproductive tract of a postpartum lactating dairy cow may be compromised in supporting early embryo development compared to nonlactating heifers. This conclusion was based on a higher proportion of embryos recovered from heifers (79.0 vs 57.2%) as well as on lower progesterone concentrations in lactating cows (d 7; 2.39 vs 5.34 ng/ml).

Walters et al. (2002) found that dairy cows produced a higher proportion of good (+) oocytes at day 119 than cows at day 32 postpartum.

Aspiration of oocytes took place in these two groups (early lactation, mid-lactation) and in the following days (twice weekly, over a 10-week period). The results showed a linear increase in the number of follicles in both groups. The number of follicles increased from 14.2 (Day 119) to 18.1 (Day 190) in mid-lactation and from 14.9 (Day 32) to 15.7 (Day 90) in early-lactation. This fact indicates that the length of postpartum could have an important effect. Most research has been focused on standard reproduction characteristics, e.g., the service period, number and quality of embryos or oocytes. Evaluation of the corpus luteum count as an important informative trait is still missing.

For these reasons, the objective of this study was to evaluate the effect of the postpartum period of different lengths (early postpartum period up to 3.5 months; middle postpartum period of 3.7–7 months; later postpartum period above 7.5 months) on the superovulation response in Holstein dairy cows determined by the number of corpora lutea and the number and quality of flushed embryos.

Materials and Methods

The sample included 55 Holstein cows (aged 1.4 to 7.8 years) superovulated at one farm in the Czech Republic during the years 2010 and 2013. The farm was located in the lowlands. Average milk production (in the 1st lactation) at the time of the experiment was as follows: 7 982 kg of milk; 4.01% of fat; 320 kg of fat; 3.30% of protein; 264 kg of protein .

Oestrus was synchronized by injection of a prostaglandine F2α analogue – oestrophan (Bioveta a.s., Ivanovice na Hane, Czech Republic). The cows were superovulated by injection of porcine pituitary gonadotropin (Pluset ®- FSHp-LHp, Laboratorios Callier, Barcelona, Spain) twice daily for 5 days at 08:00 and 20:00 h given in decreasing doses starting with doses of 150 IU follicle stimulating hormone (FSH) +150 IU luteal hormone (LH) in the morning on day 11 to 50 IU FSH+50 IU LH in the evening on day 15 of the oestrous cycle. On day 13, oestrophan was administered for luteolysis. Insemination was performed × 4 with frozen-thawed semen at

12 h intervals started at 12 h after detection of the standing oestrous. Doses of semen from one sire produced in one batch were used. Embryo recovery was performed on day 7 after the first insemination by a standard non-surgical technique to flush out the uterine horns. Uterine flushing was conducted with a complete flush solution (Bioniche, Belleville, Ontario, Canada) using a silicone two-way Foley catheter (Minitüb GmbH, Tiefenbach, Germany). Flushed ova and embryos were transferred to the holding medium - phosphate buffered solution (PBS) with 20% foetal calf serum (FCS, Gibco BRL, USA) and assessed using a stereomicroscope. The embryos were evaluated according to their stage of development as transferable (i.e., morulas, blastocysts) or non-transferable (degenerated and unfertilized).

To determine the number of corpora lutea (CL), both ovaries were examined with sonograph Tringa Linear (Canmedical, Canada).

To evaluate the results of superovulation, the animals were divided at the start of synchronization and subsequent superovulation into three groups according to the length of the postpartum season (EPP – early postpartum period to 3.5 months; MPP – middle post-partum period 3.7–7 months; LPP – later postpartum period above 7.5 months after calving). The experimental cows were divided into groups according to the postpartum periods.

The PROC GLM of SAS® with fixed effect (breeding value of milk production) was applied to all data. Breeding values of milk production were based on the actual results from January 2015. The use of breeding values was convenient because the breeding value is the key indicator reflecting the individuals in production. Breeding values varied from -253 to +1 155.

The effects of the postpartum period were estimated from the model as follows:

$y_{ij} = \mu + a_i + b\times(BVM) + e_{ij}$

where:

y_{ij} - corrected value (dependent variable) represented the number of corpora lutea, transferable embryos, morulae, blastocysts, total number of embryos and embryo recovery,

μ – mean value

a_i – fixed effect of postpartum period (i = 1, n = 19; i = 2, n = 20; i = 3, n = 15);

$b\times(BVM)$ – regression on the breeding value for milk production

e_{ij} – residual error

Differences between the estimated variables were tested at the level of significance $P < 0.05$ (a; b) or $P < 0.01$ (A; B).

Results

The results (Table 1) show significant differences ($P < 0.05$–0.01) between the three postpartum period groups (EPP – early postpartum period to 3.5 months; MPP – middle postpartum period of 3.7–7 months; LPP – later postpartum period above 7.5 months after calving) and the superovulation response – represented by the number of corpora lutea (4.6; 7.4; 10.8) calculated as the regression analysis with fixed effect of the breeding value for milk production.

The trend (Fig. 1) to higher counts of corpora lutea after superovulation treatment in the later postpartal period (LPP) than in early lactation (EPP) also corresponds with the total count of embryos (3.2; 2.9; 6.5) and with the morula counts too (1.2; 1.8; 3.5). The results also showed significantly ($P < 0.05$) higher counts of embryos (better results) in the later (LPP) than in the early (EPP or MPP) postpartum period.

In terms of quality of the embryos (transferable embryos), significantly better results were found in the later postpartal period than in the middle or early postpartum period (1.8; 1.7; 4.4; $P < 0.05$). On the other hand, we found no significant differences between blastocyst counts (1.9; 0.6; 1.5) or embryo recovery (56.8; 34.3; 53.7%).

We also calculated the coefficient of determination (representing the share of explained variance), which was the greatest number of corpora lutea ($r^2 = 0.41$). For the other traits there was a coefficient of determination between $r^2 = 0.20$–0.29 (Table 2).

Discussion

Generally, the results show that the superovulation response (represented by the number of corpora lutea) was significantly greater during the time period with lower loading of the organism in terms of milk production (LPP). There was a significantly lower response of

Table 1. Influence of length of the postpartal period on ovarian activity, yield and quality of the flushing embryos.

Postpartum period	Corpus luteum (n)	Total embryos (n)	Embryo recovery (%)	Transferable embryos (n)	Morulae (n)	Blastocysts (n)	Fragments (n)
	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE
EPP	4.6 ± 1.11^{A}	3.2 ± 1.10^{a}	56.8 ± 12.18	1.8 ± 0.71^{a}	1.2 ± 0.62^{a}	1.9 ± 0.84	1.2 ± 0.45
MPP	7.4 ± 1.10^{a}	2.9 ± 1.03^{b}	34.3 ± 11.40	1.7 ± 0.74^{b}	1.8 ± 0.58	0.6 ± 0.79	0.7 ± 0.45^{a}
LPP	$10.8 \pm 1.46^{A,a}$	$6.5 \pm 1.39^{a,b}$	53.7 ± 15.32	$4.4 \pm 0.93^{a,b}$	3.5 ± 0.78^{a}	1.5 ± 0.85	2.1 ± 0.59^{a}

EPP – early postpartum period (up to 3.5 months);
MPP – middle postpartum period (3.7–7 months);
LPP – later postpartum period (above 7.5 months)
The same superscript within the column means significant differences - $P < 0.05$ (a; b) or $P < 0.01$ (A; B)

the organism to superovulation during the first phase of lactation (EPP) than in the period with less energy-loading. These findings generally agree with Makarevich et al. (2016).

Similar findings were reported by Walters et al. (2002). These authors also showed increased number of follicles from 14.2 (Day 119) to 18.1 (Day 190) in middle-lactation, whereas in early-lactation the number of follicles increased from 14.9 (Day 32) to 15.7 (Day 90). In agreement with our study, the authors reported a higher percentage of good (+) oocytes at day 119 (4.1 ± 0.9%) than in cows at day 32 postpartum (1.6 ± 0.9%) (Walters et al. 2002).

In agreement with our findings, Kendrick et al. (1999) presented that oocyte number increased (linearly) from days 30 to 100 postpartum. At the same time the authors found that the cows produced more good oocytes when they were fed high energy diets.

Lucy et al. (1991) also found a significant relation between energy balance and follicular and ovarian function in the postpartum period in Holstein cows. The authors report that the number of large follicles (from 10 to 15 mm) detected by ultrasonography increased when the energy balance of cows increased. At the same time with the increasing energy balance, the number of small-sized follicles (from 6 to 9 mm) in Holstein cows decreased (Lucy et al. 1991).

Snijders et al. (2000) studied the effect of genetic merit (milk yield, body condition) on *in vitro* oocyte development in dairy cows. The authors found that fewer blastocysts and lower cleavage was conected with a high genetic merit of cows. On the other hand, Matoba et al. (2012) found a positive association between the milk yield and bodyweight with regard to the recovered number of oocytes. The authors did not find an effect of the lactation metabolic stress on oocytes.

De Bie et al. (2016) also hypothesized that negative energy balance has a negative effect on oocyte quality in Holstein Friesian cows. The authors report that the addition of β-carotene can improve β-carotene availability in the oocytes and hereby the oocyte quality in dairy cows. Beran et al. (2012) reported a significant effect of the sire on fertilization ability as well as pregnancy rate of cows.

Milk production is generally associated with high energy demands on animals. This is reflected in reproduction, as reported by a number of authors who describe poorer results in high production in contrast to low-lactating cows. Sartori et al. (2002) found lower embryo quality in lactating cows compared to dry cows (3.1 vs 2.2) and also comparing lactating cows and non-lactating heifers (3.8 vs 2.2). Rizos et al. (2010) also report more embryos recovered from non-lactating heifers than from lactating cows (79.0 vs 57.2%). A lower proportion of embryos reaching the blastocyst stage in all lactating cows (4%) compared to 23.2% in non-lactating heifers was also described by Leroy et al. (2005).

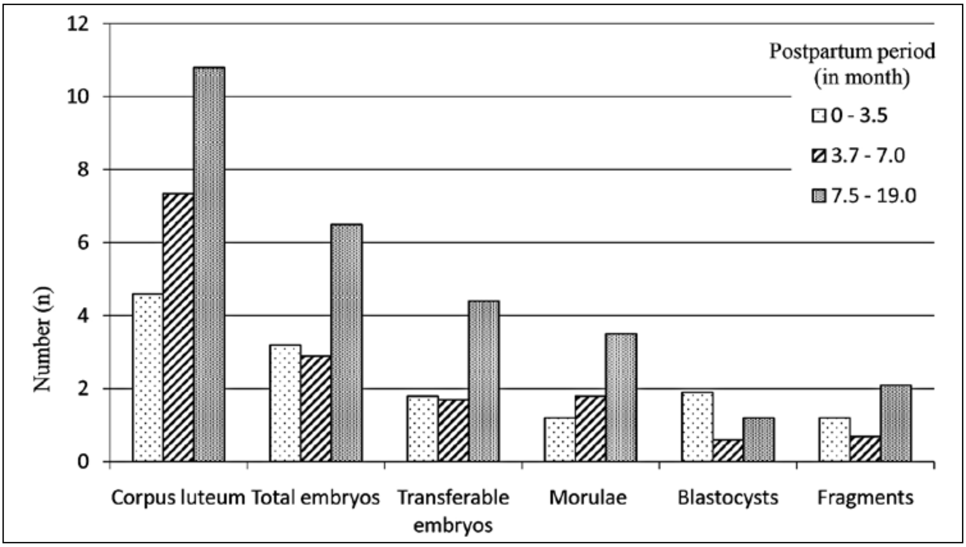

Fig. 1. Relationship of different postpartum period lengths and reproduction in dairy cows.

They determined significantly reduced embryo quality in lactating cows compared to non-lactating ones as well.

Early lactation is a period characterised by an intensive increase in milk production and high energy demands on the organism. For this reason, this period is associated with negative energy balance (Roche et al. 2007), fat mobilisation and a decrease in the body condition score (BCS). In this way, the level of metabolism significantly influences reproduction as an energy deficit which can lead to infertility (Villa-Godoy et al. 1988).

The aim of the present study was to evaluate the reproduction efficiency of cows in various postpartal periods with significantly different metabolic loads. The results demonstrate that the effects of the postpartal period on reproduction are very important; e.g., in terms of effective timing of embryo transfer, when in periods with a lower metabolic load of the organism (later postpartum period), higher ovarian activity and embryo yield can be expected than in the early lactation periods. The energy balance of the organism is also very important for the right timing of insemination.

Table 2. The coefficients of determination and significance of various factors included in the model.

Trait	Model		Time after birth		Breeding value of milk production	
	r^2	*P*	F-test	*P*	F-test	*P*
Number of corpus luteum	0.41	0.007	5.93	0.008	3.87	0.061
Number of fragments	0.29	0.047	1.55	0.232	8.34	0.008
Transferable embryos	0.23	0.126	3.03	0.069	1.17	0.292
Morulae	0.20	0.164	2.77	0.085	0.43	0.519
Blastocysts	0.20	0.558	0.60	0.568	1.74	0.22
Total embryos	0.24	0.101	2.41	0.114	4.08	0.056
Embryo recovery (%)	0.10	0.499	0.97	0.395	1.28	0.271

In conclusion, significant differences in ovarian activities after superovulation represented by numbers of corpora lutea in relation to the length of the postpartum period were determined. The results show that the superovulation response represented by the number of corpora lutea as well as by the number and quality of embryos was significantly higher during a lower load of the organism in terms of milk production.

Acknowledgements

This work was supported by “S” grant of MSMT CR and the research project NAZV QI91A061.

References

Bauman DE, Currie WB 1980: Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J Dairy Sci **63**: 1514-1529

Beran J, Stádník L, Bezdíček J, Louda F, Čítek J, Ducháček J 2012: Effect of sire and extender on sperm motility and share of live or dead sperm in bulls’ fresh ejaculate and in AI doses after thawing. Arch Tierz **55**: 207-218

Beran J, Stádník L, Ducháček J, Okrouhlá M, Doležalová M, Kadlecová V, Ptáček M 2013: Relationships among the cervical mucus urea and acetone, accuracy of insemination timing and sperm survival in Holstein cows. Anim Reprod Sci **142**: 28-34

Bezdíček J, Šubrt J, Filipčík R, Bjelka M, Dufek A 2007: The effects of inbreeding on service period and pregnancy length in Holsteins and Czech Fleckviehs after the first calving. Arch Tierz **50**: 455-463

Bezdíček J, Šubrt J, Filipčík R 2008: The effect of inbreeding on milk traits in Holstein cattle in the Czech Republic. Arch Tierz **51**: 415-425

Bezdíček J, Stádník L, Makarevich A, Kubovičová E, Louda F, Hegedüšová Z, Holásek R, Beran J, Nejdlová M 2014: Effect of inbreeding on yield and quality of embryos recovered from superovulated Holstein cows. Turk J Vet Anim Sci **38**: 681-685

Butler WR 2000: Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci **60-61**: 449-457

DeBie J, Langbeen A, Verlaet AAJ, Florizoone F, Immig I, Hermans N, Fransen E, Bols PEJ, Leroy JLMR 2016: The effect of a negative energy balance status on β-carotene availability in serum and follicular fluid of nonlactating dairy cows. J Dairy Sci **99**: 5808-5819

Kendrick KW, Bailey TL, Garst AS, Pryor AW, Ahmadzadeh A, Akers RM, Eyestone WE, Pearson RE, Gwazdauskas FC 1999: Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. J Dairy Sci **82**: 1731-1741

Loker S, Miglior F, Bohmanova J, Jamrozik J, Schaffer LR 2009: Phenotypic analysis of pregnancy effect on milk, fat, and protein yields of Canadian Ayrshire, Jersey, Brown Swiss, and Guernsey breeds. J Dairy Sci **92**: 1300-1312

Leroy JLMR, Opsomer G, De Vliegher S, Vanholder T, Goossens L, Geldhof A, Bols PEJ, de Kruif A, Van Soom A 2005: Comparison of embryo quality in high-yielding dairy cows, in dairy heifers and in beef cows. Theriogenology **64**: 2022-2036

Lucy MC 2001: Reproductive loss in high-producing dairy cattle: Where will it end? J Dairy Sci **84**: 1277-1293

Lucy MC, Staples CR, Michel FM, Thatcher WW 1991: Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J Dairy Sci **74**: 473-482

Maillo V, Rizos D, Besenfelder U, Havlicek V, Kelly AK, Garrett M, Lonergan P 2012: Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows. J Dairy Sci **95**: 3865-3876

Makarevich AV, Stádník L, Kubovičová E, Hegedüšová Z, Louda F, Beran J, Nejdlová M 2016: Quality of preimplantation embryos recovered from dairy cows in relation to their body condition. Zygote **24**: 378-388

Matoba S, O’Hara L, Carter F, Kelly AK, Fair T, Rizos D, Lonergan P 2012: The association between metabolic parameters and oocyte quality in postpartum Holstein dairy cows. J Dairy Sci **95**: 1257-1266

Rizos D, Carter F, Besenfelder U, Havlicek V, Lonergan P 2010: Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. J Dairy Sci **93**: 1022-1029

Roche JR, MacDonald KA, Burke CR, Lee JM, Berry DP 2007: Associations among body condition score, body weight, and reproductive performance in seasonal-calving dairy cattle. J Dairy Sci **90**: 376-391

Royal MD, Darwash AO, Flint APF, Webb R, Woolliams JA, Lamming GE 2000: Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci **70**: 487-501

Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC 2002: Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci **85**: 2803-2812

Snijders SE, Dillon P, O’Callaghan D, Boland MP 2000: Effect of genetic merit, milk yield, body condition and lactation number on in vitro oocyte development in dairy cows. Theriogenology **53**: 981-989

Stádník L, Louda F 1999: The effect of genetic parameters of sire in France on the performance and reproduction of daughters imported to the Czech Republic and calving here. Czech J Anim Sci **44**: 433-439

Stádník L, Louda F, Ježková A 2002: The effect of selected factors at insemination on reproduction of Holstein cows. Czech J Anim Sci **47**: 169-175
Stádník L, Ducháček J, Beran J, Toušová R, Ptáček M 2015: Relationships between milk fatty acids composition in early lactation and subsequent reproductive performance in Czech Fleckvieh cows. Anim Reprod Sci **155**: 75-79
Villa-Godoy A, Hughes TL, Emery RS, Chapin LT, Fogwell RL 1988: Association between energy balance and luteal function in lactating dairy cows. J Dairy Sci **71**: 1063-1072
Walters AH, Bailey TL, Pearson RE, Gwazdauskas FC 2002: Parity-related changes in bovine follicle and oocyte populations, oocyte quality, and hormones to 90 days postpartum. J Dairy Sci **85**: 824-832
SAS (2009): SAS/STAT® 9.1. User's Guide. Cary, NC: SAS Institute Inc. 5121 p.