Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2014) 38: 681-685
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doi:10.3906/vet-1403-95

Effect of inbreeding on yield and quality of embryos recovered from superovulated Holstein cows

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Received: 31.03.2014 • **Accepted:** 20.06.2014 • **Published Online:** 24.10.2014 • **Printed:** 21.11.2014

Abstract: The aim of this study was to evaluate the effect of inbreeding on the yield and quality of bovine embryos. The data were obtained from 125 Holstein cows superovulated on 3 farms in the Czech Republic. In total, 125 flushings of embryos were analyzed, from which 761 embryos were obtained. Of all donors, 36 cows were inbred (199 embryos) and 89 cows were noninbred (562 embryos). The inbred cows were divided into 2 groups according to their inbreeding coefficient (F_X) level. The low F_X group (F_X 1.26%–1.56%) had 23 cows and 147 embryos, and the high F_X group (F_X 3.1%–25%) was represented by 13 cows and 52 embryos. The average value of the F_X coefficient in inbred cows was 6.552%. The data were subsequently analyzed using PROC GLM of SAS as descriptive statistics and t-tests (SAS/STAT® 9. 1., 2009). All evaluated traits in inbred cows had worse results than noninbred cows. Although there were poorer results for inbred versus noninbred cows, the differences were not great at lower F_X values and were not statistically significant. Important and significant differences ($P \leq 0.05$) were found in the proportion of transferable embryos and unfertilized oocytes at higher rates of F_X.

Key words: Holstein, inbreeding, embryos

1. Introduction

Reproduction of cows is a crucial economic factor responsible for the transfer of genetic information to progeny. Fertility can be evaluated using a wide range of indicators (1) closely linked to the activity of the ovaries and production of oocytes (2). These typical quantitative traits are affected by a number of genetic (3–5) as well as environmental factors, including the nutritional status and energy balance (6,7), level and quality of milk production (8), hormonal status of the organism (9), body condition (10), and overall health of the animal (11). One very important genetic factor for quantitative traits is the level of inbreeding (F_X).

Studies of different cattle populations have demonstrated the importance of monitoring inbreeding. Inbreeding depression has been evaluated and confirmed in small cattle populations (12,13) as well as in breeds with large worldwide numerical distribution. The influence of inbreeding depression has been confirmed mainly on traditional traits, such as length of service period (14–16), number of services and conception rate (14), and length of the first calving interval (17). Alvarez et al. (18) determined a lower number of transferable embryos in cows with F_X = 9.0%–30.0% compared to those with F_X = 0%–8.9% ($P < 0.05$). However, no linear or quadratic effects of inbreeding on total or nontransferable embryos were found. They stated that this depression could relate to ovarian dysfunction. According to Szabari et al. (29), the greatest problem in embryo recovery is the wide variability of ovarian responses to hormonal treatment, which significantly increases the total cost per embryo obtained. On the other hand, Alvarez et al. (20) found no differences in pituitary gonadotropins or ovarian steroids in highly inbred versus noninbred superovulated cows.

The number of flushed dairy cow donors is continuously increasing worldwide (21). Therefore, generally, understanding all the factors influencing embryo production is essential for breeding and achieving a satisfactory level of economical effectiveness of embryo recovery and transfer (22).

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The aforementioned studies provide a basis for hypothesizing that different percentages of inbreeding in dairy cows could cause significant differences in ovarian responses to superovulation protocols and subsequently in the yield and quality of embryos. Currently, very little research is focused on the yield and quality of bovine embryos in relation to inbreeding. Thus, the aim of this study was to evaluate the effect of different amounts of inbreeding on the yield and quality of embryos obtained from superovulated Holstein cows.

2. Materials and methods

The data were obtained from 125 Holstein cows superovulated on 3 farms in the Czech Republic during 2010 and 2011. The superovulation was administered over the 2 years, and individual observations were divided according to season.

Estrus was synchronized by injection of a PGF 2α analogue (Oestrophan; Bioveta a.s., Czech Republic). The cows were superovulated by injection of porcine pituitary gonadotropin (Pluset - FSHp-LHp, Laboratorios Callier, Spain) twice daily for 5 days at 0800 and 2000 hours, given in a decreasing dosage rate starting with doses of 150 IU FSH + 150 IU LH in the morning on day 11 to 50 IU FSH + 50 IU LH in the evening on day 15 of the estrous cycle. On day 13, Oestrophan was administered for luteolysis. Insemination was performed 4 times with frozen-thawed semen at 12-h intervals, beginning at 12 h after detection of the standing estrus. Doses of semen from one sire produced in one batch were used.

Embryo recovery was performed on days 6 and 7 (a day after the first insemination) with a standard nonsurgical technique to flush out the uterine horns. Uterine flushing was conducted with a complete flush solution (Bioniche, Canada) using a silicone 2-way Foley catheter (Minitüb GmbH, Germany). Flushed ova and embryos were transferred to the holding medium, a phosphate-buffered solution with 20% fetal calf serum (Gibco BRL), and assessed using a stereomicroscope. The embryos were evaluated according to their stage of development as transferable (i.e. morulas, blastocysts) or nontransferable (degenerated and unfertilized).

For this study, cows were donors of embryos specifically used for embryo transfer. Therefore, the database included only donors with an excellent pedigree (father and mother) and high breeding value for kilograms of milk (relative breeding value above 125, calculated January 2013). The aim was to create a genetically uniform group. This study included only cows with a complete pedigree to the fifth generation and records for season and year of embryo flushing and farm. The level of inbreeding (inbreeding coefficient F_X) was calculated according to Wright (1922). The B-calc software (23) was used for evaluation of F_X.

$$\mathbf{F_X = \Sigma\, 0.5^{n + n' + 1}(1 + F_a)}$$

Σ = sum over all paths through to common ancestor

n = number of generations from the sire to common ancestor

n′ = number of generations from dam to common ancestor

F_a = inbreeding coefficient of common ancestor

The inbreeding coefficients were in the range F_X = 1.26%–25%. Average value of F_X in inbred cows was 6.552%. In total, 761 embryos were analyzed from 125 flushings. Of all donors, 36 were inbred (199 embryos) and 89 were noninbred cows (562 embryos). The inbred cows were divided into 2 groups according to F_X values (low and high F_X groups) and compared with noninbred cows (F_X = 0). The low F_X group (F_X 1.26%–1.56%) included 23 cows and 147 embryos, and the high F_X group (F_X 3.1%–25%) was represented by 13 cows and 52 embryos.

Regarding number and quality of flushed embryos, the following variables in inbred and noninbred cows were evaluated: number of embryos, number of transferable embryos, transferable embryos (in %), degenerated embryos (in %), and unfertilized oocytes (in %). Calculated percentage rates were related to the number of embryos in inbred cows (199) or in noninbred cows (562). The data were subsequently analyzed using PROC GLM of SAS, using descriptive statistics and t-tests (24).

Selected effects, which according to the model used for ovarian activity significantly contributed to the results, were included in the final calculation. Data were classified according to the combined effect of herd-year-season (HYS). The effects of inbreeding and other factors were estimated from the model as follows:

$$\mathbf{Y_{ijk} = \mu + HYS_i + F_{Xj} + e_{ijk},}$$

where Y_{ijk} is the observed value of the dependent variable (count of embryos, transferable embryos in count and %, degenerated embryos in %, unfertilized oocytes in %); μ is average value of dependent variable; HYS_i is fixed effect of i herd-year-season of flushing (3 herds; 2 years, 2010 and 2011; 3 flushing seasons, November to March; April to May and September to October; June to August, were considered); F_{Xj} is the fixed effect of j class of inbreeding (F_X = 0, n = 89; F_X = 1.256%–1.564%, n = 23; F_X = 3.1%–25%, n = 13); e_{ijk} is residual error.

The differences among groups in the variables estimated were tested at the significance levels $P < 0.05$ and $P < 0.01$. Correlation analyses between the level of inbreeding and individual dependent variables were also part of the evaluation, including calculation of determination coefficients R^2 indicating a proportion of explained variability and P-levels of statistical significance for the individual mutual relations evaluated.

The occurrence of higher F_X coefficients ($F_X \geq 12.5\%$) is very exceptional in the Czech Republic. These are random errors in the population, caused by unsuitable selected sires.

3. Results

Table 1 contains the basic statistical characteristics of the embryos from inbred and noninbred cows. The counts of embryos, taking into account the combined effect (herd, year, and season of flushing) according to the group F_X coefficient, are presented in Table 2. The results depict the distribution of total number of embryos. From this table, inbred animals with a lower coefficient F_X (from 1.26% to 1.56%) had a very small and insignificant difference in quality and proportion of transferable embryos compared with group $F_X = 0$. At greater inbreeding coefficients (3.1%–25%), the reduction in embryo quality increased. The explanatory value of the whole model is documented by the significant values of determination coefficient $R^2 = 0.2424$ and $P < 0.001$.

The results of correlation and regression analysis (without accounting for the combined effect) are shown in Table 3. These analyses reflect the relationship among F_X (in %), yield, and quality of embryos in superovulated Holstein cows. The only significant correlation was found between F_X and the proportion of degenerated embryos (in %). The highest coefficient of determination was $R^2 = 0.1883$. This coefficient indicates a lower share of explained variability, although its value corresponds to the common range detected for reproductive traits (3,17). The results of the regression analysis indicated that each 1% increase in F_X increased the proportion of degenerated embryos by 2.23% ($P < 0.05$). The regression coefficient $R = 0.4339$ shows the moderate dependence between these observed variables.

4. Discussion

Numerous studies in Holstein cows indicate that a high inbreeding coefficient downgrades traditional reproduction traits, e.g., age at first calving, conception rate, calving interval, and other reproductive traits (25,26). On the other hand, few publications have evaluated the relationship of inbreeding and quantity, or eventual quality, of embryos obtained from superovulated cows. The results in this study are comparable with those reported by Alvarez et al. (20). Those authors evaluated the effect of inbreeding depression on ovarian activity in superovulated Mantiqueira breed cows. The regression equation was significant for transferable ($R^2 = 0.91$) but not for nontransferable ($R^2 = 0.13$) or total ($R^2 = 0.63$) embryos. In a subsequent study (18), Alvarez et al. described a decrease in the count of transferable embryos in the inbreeding group: F_X = 3%–5.9%, F_X = 6%–8.9%, and F_X = 9%–30%. The counts of transferable embryos in these groups were 7.0, 5.8, and 3.5, respectively. Similarly, Van Raden and Miller (27) found that increased inbreeding of embryos had a negative impact on reproduction. These findings are consistent with the present results as well. Although different types of animals differ in sensitivity to inbreeding depression, the present results are comparable with the results of the negative effects of inbreeding on ovulatory activity in turkeys (28), Japanese quail (29), and hens (30).

Table 1. Basic statistical analysis of embryo production in inbred and noninbred cows.

	Inbred cows					Noninbred cows					P	P (F-ratio)
	n	$\bar{x}$	min.	max.	s_d	n	$\bar{x}$	min.	max.	s_d		
Number of embryos (n)	199	5.53	0	16	3.86	562	6.32	0	21	5.12	0.4074	0.0627
Transferable embryos (n)	141	3.92	0	15	3.46	376	4.22	0	20	4.06	0.6895	0.2919

Table 2. PROC GLM analysis of embryos proportion in groups according to level of Fx.

Variable	Group of Fx					
	Fx = 0 (n = 562)		Fx = 1.26%–1.56% (n = 147)		Fx = 3.1%–25% (n = 52)	
	LSM	SE	LSM	SE	LSM	SE
Transferable embryos (in %)	71.77[a]	3.64	68.27	6.57	55.13[a]	8.88
Degenerated embryos (in %)	12.46	2.92	14.89	5.26	16.06	7.10
Unfertilized oocytes (in %)	15.77[a]	3,06	16.84	5.51	28.81[a]	7.45

The same superscript letters means significant difference among columns, P < 0.05.

Table 3. Basic correlation and regression statistics among the coefficient F_x (in %) and the yield and quality of embryos in superovulated Holstein cows.

		R	Multiple R^2	Adjusted R^2	Rxy	P-level
F_X vs.	Number of embryos (n)	0.2818	0.0794	0.0523	–0.2085	0.0959
	Transferable embryos (n)	0.2673	0.0715	0.0442	–0.1773	0.1149
	Transferable embryos (in %,)	0.2753	0.0758	0.0469	–1.6857	0.1151
	Degenerated embryos (in %)	0.4339	0.1883	0.1629	2.2347	0.0104
	Unfertilized oocytes (in %)	0.1055	0.0111	–0.0198	–0.5489	0.5528

In conclusion, based on the results of this work and financial demands for embryo transfer, we can recommend eliminating dairy cows with F_X values of 3% and higher from the group of donors in the future, because higher F_X values resulted in a significant decline in transferable embryo share. This simple principle could contribute to greater effectiveness of the mentioned biotechnological technique and yield the highest possible number of transferable embryos. These findings have significant importance in relation to the present percentage of inbreeding in Holstein dairy cows as well as the number of embryo transfers performed worldwide.

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

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