Analysis of prolificacy in sows of hyperprolific lines of Large White breed

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ABSTRACT: The objective of the paper was to evaluate the results of reproductive performance of sows in some elite breeding herds of the Large White breed included in experimental herds for the production of hyperprolific lines of dam breeds. The set consisted of 98 sows with the known genotypes of *ESR*, *FSH*β and *PRLR* genes. The dendrogram shows that on the basis of their genetic outfit the sows can be divided into two clusters. The first cluster can be described as a cluster with marked dominance of HPL sows and the second cluster with marked dominance of the sows of basic herd. The first cluster consisted of individuals in which the preferred genotype *AA* of *PRLR* gene was not detected. As for *FSH*β gene, the beneficial genotype *BB* was found out in 74.10% of sows. In *ESR* gene the beneficial genotype *DD* was recorded only in 11.10% of individuals. On average for the lifetime performance they delivered by 2.08 piglets more in all born piglets and by 1.96 piglets more in live-born piglets per litter. Differences in the reproductive traits between HPL sows and the sows of basic herd in the first cluster were statistically highly significant. On the contrary, genotype *AA* of *PRLR* gene was identified in all sows of the second cluster, 61.36% of animals possessed beneficial genotype *BB* of *FSH*β gene. As for *ESR* gene, beneficial genotype *DD* was identified within the whole cluster in 31.82% of sows. In lifetime performance the HPL sows had on average by 1.10 individuals more in all born piglets and by 1.01 more in live-born piglets (statistically significantly higher values). The unambiguous expression of a positive effect of preferred genotypes of selected candidate genes failed to be confirmed by the results of statistical analyses testing the associations of candidate genes for pig reproduction with selected parameters of breeding value and prolificacy of sows.

Keywords: sow; Large White; hyperprolific line; reproduction; *ESR*; *FSH*β; *PRLR*

Selection for an increase in prolificacy of sows is difficult due to low heritability. One of the possibilities is production of hyperprolific lines of dam breeds that is closely connected with the use of BLUP-animal model and with the application of molecular genetics knowledge.

The objective of the paper was to evaluate the results of reproductive performance of sows in some elite breeding herds of the Large White breed included in experimental herds for the production of hyperprolific lines of dam breeds and to determine the proportions of genotypes of selected genes in order to test their effect on reproductive traits.

Hyperprolific lines originate by selection of sows with the highest breeding value for reproduction. The sow performance should be as follows: on average 12 live-born piglets per litter in lifetime performance, minimum number of teats 14 in the 7/7 position and maximum backfat thickness determined in performance testing 12 mm. Beneficial alleles of *ESR* gene are desirable in selected sows (but they are not a condition). Performance testing is carried out in accordance with the standard ČSN 46 6164; individual birth weight of piglets, maternal behaviour of sows and parturition length are also determined (methodical instructions of Pig Breeders Association).

In France the method of hyperprolific lines in pig breeding contributed to an increase in the genetic potential of pigs to a high international level. The

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method consists in selection of sows with extremely high performance in reproductive traits (number of live-born piglets) in connection with the high selection level. Targeted mating of sons after especially prolific mothers and unrelated highly prolific sows resulted in an increase in the frequencies of some alleles that are related with reproductive traits.

Oestrogen receptor gene, prolactin receptor gene and gene of follicle-stimulating hormone are the best-known candidate genes for pig reproduction.

Oestrogens are sex steroid hormones of mammals. They stimulate cell proliferation and growth of the tissues related to reproduction. For their function the activation of oestrogen receptor is necessary that mediates information between the cytoplasm and DNA of the cell nucleus. Dvořák (1999) reported that two genes of oestrogen receptor were described: *ESR*α localised on chromosome 1 and *ESR*β localised on chromosome 7. In the gene for oestrogen receptor α three polymorphic loci were identified that can be detected by restriction enzymes *Ava*I, *Msp*AI and *Pvu*II. Point mutations in *ESR* gene in Chinese breeds of pigs by means of endonuclease *Pvu*II were described by Rothschild *et al.* (1991). This polymorphic system is denoted *ESR2*. According to the original methodical technique allele *Pvu*II$^-$ is designated as allele *A*, and allele *Pvu*II$^+$ as allele *B*. In the Czech Republic the alleles are designated *C* and *D*, respectively. To test this polymorphism Rothschild *et al.* (1995) worked out a genetic test based on the combination of PCR and restriction fragment length polymorphism (RFLP). Drögemüller *et al.* (1997) identified other two polymorphisms (*ESR*1 and *ESR*3) using the PCR-RFLP method and restriction endonucleases *Ava*I and *Msp*AI.

Rothschild (1996) tested the role of oestrogen receptor gene in litter size. Preferred allele *B* was associated with an increase in the number of piglets in the Large White breed by 0.4–0.5 piglets per litter. Urban *et al.* (2002) reported the following frequency of genotypes of *ESR* gene in the group of sows of Large White breed: 29% *CC*, 44% *CD* and 27% *DD*. They determined a significant association of ESR gene with the number of all born piglets, number of live-born piglets and mortality rate of piglets in parity 1.

The follicle-stimulating hormone is glycoprotein produced by the anterior lobe of the pituitary gland. It has subunits α and β encoded by other genes. FSH acts on follicle cells. Subunit β is species specific, it has a specific biological function and is encoded by *FSH*β gene (Mellink *et al.*, 1995). Li *et al.* (1998) confirmed the association of *FSH*β gene with litter size. Chen *et al.* (2001) stated that in sows of the combined genotype *BBBB* the number all born piglets was higher by 1.85–3.01 and the number of live-born piglets was higher by 2.0–3.0 compared to sows of *ABAA* genotype. They drew a conclusion that the effect of *ESR* and *FSH*β genes on litter size contributed to the improvement of reproductive traits by marker-assisted selection (MAS) and that the introgression of the beneficial gene expression into productive lines of pigs in which the expression of alleles was not presented improved commercially important reproductive traits.

Prolactin receptor gene is examined as a candidate gene for litter size on the basis of its important role in prolactin metabolism (Kelly *et al.*, 1991). According to Dvořák (1999) the average effect of allele *B* of *PRLR* gene is by 0.25 piglets more in parity 1. Vincent *et al.* (1998) tested *PRLR* in five lines of the PIC company. Two lines came from Large White breed, one line from Landrace breed and the other two lines from the crossing of Duroc × Large White and Large White × Meishan breeds. The *AA* genotype sows of the line of Large White breed had by 0.66 piglets more per litter than heterozygous *AB* sows. Rens and Van der Lende (2002) reported that the gilts of *AA* genotype of *PRLR* gene delivered a higher number of all born piglets ($P = 0.047$) and live-born piglets ($P = 0.062$) than the gilts of *BB* genotype. For *AA*, *AB* and *BB* genotypes their respective values for the number of all born piglets were 11.4; 10.8 and 8.8 piglets and for the number of live-born piglets 11.1; 10.5 and 8.7 piglets. Kmiec *et al.* (2001) also demonstrated a significantly higher number of piglets typical of sows of *AA* genotype of *PRLR* gene.

MATERIAL AND METHODS

Material

Data from the performance testing of reproductive traits of sows of the Large White breed from three elite breeding herds were used for this study. The set consisted of 98 sows with the known genotypes of *ESR*, *FSH*β and *PRLR* genes born in 1995–2002. Fifty-one sows were included in the hyperprolific line (HPL) while the basic herd (BH) comprised 47 sows.

For the analysis of reproduction we used these parameters:
– total breeding value in CZK calculated from the formula HPL – TBVhpl
– breeding value for reproduction (number of piglets) – BVrep
– average number of all born piglets per litter in lifetime performance and/or in parity 1, in parity 2 and higher (number of piglets) – All P and/or All 1 and All P2
– average number of live-born piglets per litter in lifetime performance and/or in parity 1, in parity 2 and higher (number of piglets) – Live P and/or Live 1 and Live P2

and three candidate genes for pig reproduction:
– oestrogen receptor (*ESR*) with alleles *C* and *D*
– follicle-stimulating hormone (*FSH*β) with alleles *A* and *B*
– prolactin receptor (*PRLR*) with alleles *A* and *B*

Genotypes were determined by molecular genetics methods PCR-RFLP in Laboratory of Applied Genetics affiliated to Mendel University of Agriculture and Forestry in Brno (LamGen). DNA was isolated from blood samples with addition of EDTA as anticoagulant.

Statistical processing

Statistical processing is based on the knowledge acquired by the authors cited by Dvořák *et al.* (1999) when allele *D* of *ESR* gene is assumed to have a positive association with litter size, and allele *A* of *PRLR* gene and allele *B* of *FSH*β gene are assumed to have a positive effect on reproductive traits.

Therefore each genotype was encoded according to the key:
1 – in the case when the "beneficial" allele was in homozygous condition
0 – in the other cases

A set of objects can be taken as a set with alternative multivariate distribution. At first, hierarchic cluster analysis was used as a statistical method. Its aim was to divide the sows into clusters (groups) with similar genetic "outfit". As the alternative distribution was used, it was necessary to choose a special measure of similarity for the construction of similarity matrix and to take into account data discontinuity in the choice of clustering method. Among the measures of similarity for binary data Sokal-Michener Simple Matching Similarity Measure was used when similarity between two objects x and y, i.e. SM (x, y), is calculated from the equation:

$$SM\,(x, y) = \frac{a + b}{a + b + c + d}$$

where: a = number of cases of positive matching, i.e. ones
d = number of cases of negative matching, i.e. zeros
b, c = number of cases of positive or negative non-matching in a contingency table that must be constructed for each pair of sows

Average Linkage (Within Group) Method was used as a clustering method.

To visualise the measures of positions exploration analysis of data was carried out in each cluster. A robust measure of position was chosen (i.e. median in connection with upper and lower quartile).

The reproductive traits of sows in the particular clusters were tested by t-test. Before, F-test was used to verify the fit of variances. As the sets were small, the results of t-test were also verified by exact non-parametric Mann-Whitney test. The results of both tests led to identical conclusions.

RESULTS AND DISCUSSION

The resultant dendrogram (Figure 1) shows that on the basis of their genetic outfit the sows can be divided into two clusters of nearly the same size. Table 1 shows descriptions of these clusters. It is evident that 55.10% of animals in total were included in the first cluster. Of them, 70.37% were HPL sows and 29.63% of sows of the basic herd. This cluster can be described as a cluster with marked dominance of HPL sows. The second cluster comprises 44.90% of animals out of the total number of analysed individuals. The ratio of HPL sows to basic-herd sows is reverse, i.e. 29.55% of HPL sows and 70.45% of basic-herd sows. It is a cluster with marked dominance of the sows of basic herd.

The first cluster (Table 1) consisted of individuals in which the preferred genotype *AA* of *PRLR* gene was not detected. As for *FSH*β gene, the beneficial genotype *BB* was found out in 74.10% of sows (almost ¾ of sows): in 78.95% of sows included in HPL and in 62.50% of sows of the basic herd. In *ESR* gene the beneficial genotype *DD* was recorded only in 11.10% of individuals: out of them

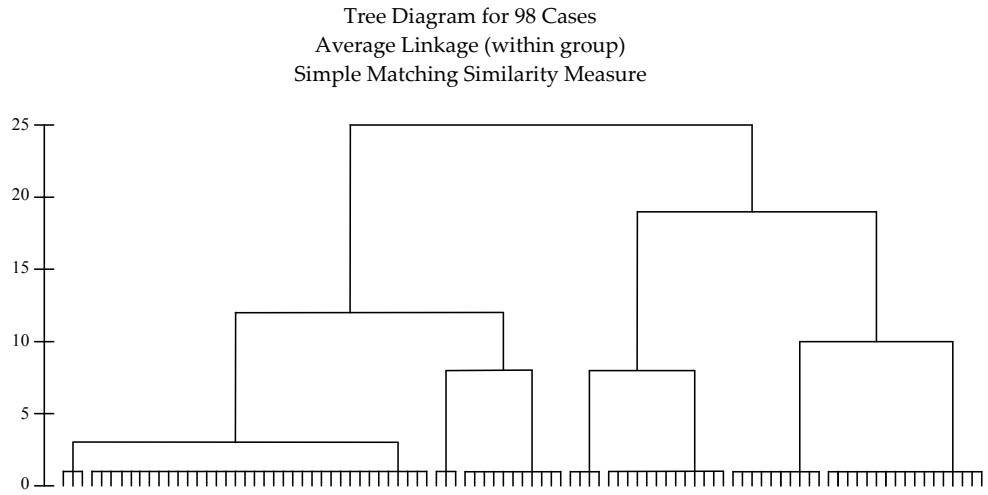

Figure 1. The resultant dendrogram

in 7.89% of HPL sows. In the sows of basic herd this proportion was higher – 18.75% of animals. Table 2 shows the basic statistical characteristics and results of tests of selected reproductive traits in the sows of HPL and basic herd included in the first cluster. Total breeding value of HPL sows was on average higher by 550 CZK than in the sows of basic herd (Figure 2). Breeding value for reproduction was higher in HPL sows by 1.02 piglets than the average value of the population achieved in the fixed period for the calculation of breeding value for reproduction. As assumed, the sows included in HPL had higher reproductive traits compared to the sows of basic herd. On average for the lifetime performance they delivered by 2.08 piglets more in all born piglets and by 1.96 piglets more in live-born piglets per litter. In parity 1 the difference was 2.09 individuals both in all born piglets and in live-born piglets, in parity 2 and higher it was 1.97 individuals in all born piglets and 1.81 individuals in live-born piglets. Differences in the given breeding values and reproductive traits between HPL sows and the sows of basic herd in the first cluster were statistically highly significant.

On the contrary, genotype *AA* of *PRLR* gene was identified in all sows of the second cluster (Table 1).

Table 1. Description of the first and second cluster

First cluster				Second cluster			
54 individuals		55.10%		44 individuals		44.90%	
Of them		out of the total	out of the cluster	of them		out of the total	out of the cluster
HPL	38 indiv.	74.51%	70.37%	HPL	13 indiv.	25.49%	29.55%
BH	16 indiv.	34.04%	29.63%	BH	31 indiv.	65.96%	70.45%
No individual possesses *PRLR* = *AA*				All individuals possesses *PRLR* = *AA*			
*FSH*β				*FSH*β			
	total	HPL	BH		total	HPL	BH
BB yes	74.10%	78.95%	62.50%	*BB* yes	61.36%	61.54%	61.29%
BB no	25.90%	21.05%	37.50%	*BB* no	38.64%	38.46%	38.71%
ESR				*ESR*			
	total	HPL	BH		total	HPL	BH
DD yes	11.10%	7.89%	18.75%	*DD* yes	31.82%	30.77%	32.26%
DD no	88.90%	92.11%	81.25%	*DD* no	68.18%	69.23%	67.74%

Table 2. Basic statistical characteristics and results of tests – the first cluster

	n		Mean		Median		*s*		*F*-test	*P*	*t*-test	*P* 1-sided
	HPL	BH	HPL	BH	HPL	BH	HPL	BH				
TBVhpl (CZK)	38	16	1 789	1 239	1 729	1 264	356	303	1.387	0.504	5.402	2E-06
BVrep (No. ±)	38	16	2.13	1.11	2.04	1.13	0.56	0.41	1.642	0.218	3.789	4E-04
All P (No.)	38	16	14.06	11.98	14.30	11.65	1.49	1.81	1.481	0.326	4.388	6E-05
Live P (No.)	38	16	13.70	11.74	14.00	11.60	1.36	1.64	1.445	0.356	4.546	3E-05
All 1 (No.)	38	16	13.71	11.63	14.00	11.00	1.89	2.00	1.118	0.750	3.645	6E-04
Live 1 (No.)	38	16	13.53	11.44	13.00	11.00	1.78	1.86	1.091	0.794	3.883	3E-04
All P2 (No.)	38	16	14.11	12.14	14.00	12.30	1.63	2.13	1.718	0.180	3.693	5E-04
Live P2 (No.)	38	16	13.67	11.85	13.50	12.00	1.48	1.89	1.642	0.218	3.788	4E-04

Almost two thirds of the sows, i.e. 61.36% of animals, possessed beneficial genotype *BB* of *FSH*β gene. The identical proportion of HPL sows and basic-herd sows had this genotype: 61.54% and 61.29%, respectively. As for *ESR* gene, beneficial genotype *DD* was identified within the whole cluster in 31.82% of sows. The respective proportions were 30.77% of HPL sows and 32.26% of basic-herd sows. The HPL sows achieved total breeding value that was statistically highly significantly higher by 283 CZK on average (Table 3, Figure 3). The table also shows that HPL sows delivered on average by 0.55 piglets more than was the average value of the population for the fixed period for the calculation of breeding value (statistically highly significantly higher values). In lifetime performance the HPL sows had on average by 1.10 individuals more in all born piglets and by 1.01 more in live-born piglets (statistically significantly higher values). In parity 1 the difference was 0.81 for all born piglets and 0.74 for live-born piglets; in parity 2 and higher the respective differences were 0.80 and 0.78. Compared to the basic-herd sows, these values of reproductive traits in parity 1 and in parity 2 and higher were not statistically significant. For comparison, Table 4 shows the results of non-parametric single tail tests including the exact ones specially constructed for small samplings. The values in the table document that neither in this case did the HPL sows have statistically significantly higher mean values of reproductive traits.

Bidanel and Ducos (1994) reported genetic improvement of litter size by 0.9 piglets per parity in hyperprolific lines of daughters after boars of the hyperprolific line of Large White breed in comparison with the normal population that achieved

Table 3. Basic statistical characteristics and results of tests – the second cluster

	n		Mean		Median		*s*		*F*-test	*P*	*t*-test	*P* 1-sided
	HPL	BH	HPL	BH	HPL	BH	HPL	BH				
TBVhpl (CZK)	13	31	1 609	1 326	1 629	1 297	327	236	1.922	0.145	3.232	0.001
BVrep (No. ±)	13	31	1.73	1.18	1.71	1.18	0.47	0.36	1.726	0.221	4.174	7E-05
All P (No.)	13	31	12.84	11.74	12.80	11.80	1.96	1.74	1.270	0.572	1.838	0.037
Live P (No.)	13	31	12.56	11.55	12.50	11.50	1.75	1.72	1.032	0.893	1.776	0.041
All 1 (No.)	13	31	12.23	11.42	13.00	11.00	2.35	1.65	2.033	0.114	1.309	0.099
Live 1 (No.)	13	31	12.00	11.26	12.00	11.00	2.27	1.69	1.804	0.187	1.196	0.119
All P2 (No.)	13	31	13.17	12.36	13.00	12.30	2.30	1.74	1.737	0.216	1.269	0.106
Live P2 (No.)	13	31	12.86	12.08	13.00	12.00	1.98	1.47	1.817	0.182	1.455	0.077

Table 4. Results of non-parametric tests

	All 1	Live 1	All P2	Live P2
Mann-Whitney U	153.0	158.0	157.0	148.5
Wilcoxon W	649.0	654.0	653.0	644.5
Z	–1.265	–1.137	–1.170	–1.394
Asymptotic *P*-value (2-sided)	0.206	0.255	0.242	0.163
Exact *P*-value (2-sided)	0.210	0.261	0.248	0.167
Exact *P*-value (1-sided)	0.105	0.131	0.124	0.084
Point Probability	0.002	0.002	0.003	0.002

genetic progress of litter size 0.2 piglets per parity in the same period. Experimental tests (Legault and Grund, 1976; Legault *et al.*, 1981) and subsequent testing among breeders (Bidanel *et al.*, 1994) confirmed that compared to the rest of the population daughters of hyperprolific boars had by 0.7–1.3 more live born piglets and by 0.5–0.9 more weaned piglets per parity. The results of Králová (2003) also proved that in lifetime performance, in parity 1 and parity 2 and higher, the sows of the hyperprolific line achieved statistically significantly higher parameters of breeding value, larger litter size in lifetime reproduction (13.62, 12.49 and 11.53 all born, live-born and reared piglets, respectively), larger litter size in parity 1 (13.21, 12.94 and 11.30 all born, live-born and reared piglets, resp.) and larger litter size in parity 2 and higher (13.86, 13.40 and 11.70 all born, live-born and reared piglets, respectively).

The unambiguous expression of a positive effect of preferred genotypes of selected candidate genes failed to be confirmed by the results of statistical analyses testing the associations of candidate genes for pig reproduction with selected parameters of breeding value and prolificacy of sows. It is to state on the basis of the statistical analyses that it is not possible to draw any general conclusions about the effect of *ESR*, *FSH*β and *PRLR* genes on the traits of litter size. Vaňo (2003) reported that the effect of *ESR* gene was different in the particular genotypes of sows and parities: the size and the (positive, negative) direction of this effect were different. Matoušek *et al.* (2003) found out significant differences between the groups of *ESR* genotypes in two elite breeding herds of Large White breed. In herd A statistically significant differences ($P \leq 0.05$, $P \leq 0.01$) were determined between *ESR* genotypes in the groups of parities 1–6 and 2–6 in the number of all born, live-born and weaned piglets. Sows with *DD* genotypes had the highest performance. In herd *B* significant differences ($P \leq 0.05$) between the genotypes were calculated in the group

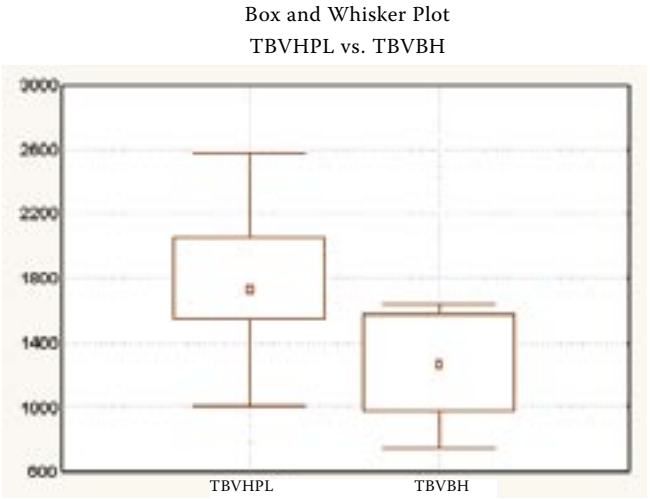

Figure 2. Total breeding values of HPL and basic-herd sows – the first cluster (in CZK)

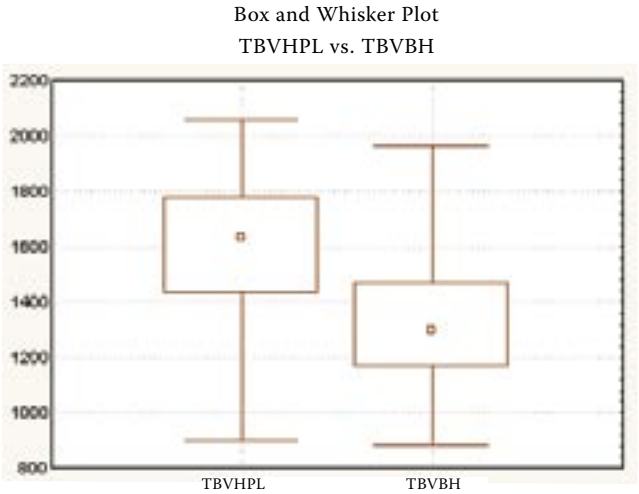

Figure 3. Total breeding values of HPL and basic-herd sows – the second cluster (in CZK)

Min–Max
25%–75%
Mean values

of parities 2–6 and in the group of parities 1–6 on the significance level ($P \leq 0.07$) in the number of all born, live-born and weaned piglets, but in favour of sows with CC genotypes. Rens *et al.* (2003) were convinced that *PRLR* gene was a candidate gene for the rate of ovulation, not for litter size. The results of Drögemüller's *et al.* (2001) study showed that the expression of alleles was different in the particular lines or populations; it could be caused by various linkages between alleles and random mutations in different lines. The results can also be explained by a high number of low-effect genes influencing the litter size. Therefore we suggest to plan selection strategy for each line separately, and possible pleiotropic effects should also be considered.

CONCLUSIONS

Differences determined between the sows of hyperprolific line and the sows of basic herd in the level of breeding values and in reproductive traits confirmed the optimally chosen levels of selection criteria for selection of sows into the hyperprolific line. The boundaries of sow selection defined in this way make it possible to form a subpopulation of hyperprolific sows with statistically significantly higher results of reproduction compared to the population of the other sows.

If we study the effect of individual genes on commercially important traits of farm animals, we should be aware of the fact that the genome of animals is a complete set realised through a branched network of interactions on different levels of biological organisation. It seems difficult to find a direct proof of the effect of a particular gene even within different populations and environmental conditions. Further studies should be aimed at the analysis of a higher number of genes in order to identify gene interactions and to describe mechanisms of gene activities.

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