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ASSOCIATION OF PGR POLYMORPHISM WITH REPRODUCTION AND PRODUCTION TRAITS IN RABBITS

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Abstract - The polymorphism and three different genotypes (AA, AG and GG) by PCR RFLP method were detected in the progesterone receptor gene (PGR) promoter in local crossbreed rabbit line. We have noted slight majority frequency of non-mutated allele G (0. 54) over allele A (0.46). Associated studies aimed at the effect of genotypes AA, AG and GG on litter size showed the highest litter size and liveborn kits in litter had does with genotype GG. The best results for milk production had the does of genotype GG followed genotype AG. In the numbers of stillborn kits were no significant differences among genotypes, however the highest pre-weaning mortality ($P<0.001$) was in heterozygous genotype AG.

Keywords - PGR, Genotype, Rabbit, Reproduction, Pre-weaning Mortality.

I. INTRODUCTION

Most reproductive traits are complex in terms of their genetic architecture [14]. Mortality and litter size is one of the most important economical traits in rabbit production. The milk production in the first 21 days of lactation has a significant impact on the growth and health of the kits and is one of the limiting factors for successful rearing during pre-weaning period.

The steroid hormone progesterone plays a central role in the reproductive events associated with pregnancy establishment and maintenance. Physiological effects of progesterone are mediated by interaction of the hormone with specific intracellular progesterone receptors (PRs) that are expressed as two protein isoforms, PR-A and PR-B. PR-A and PR-B are functionally distinct mediators of progesterone action in vivo and should provide suitable targets for generation of tissue-selective progestins [10]. PR-A is necessary and sufficient to elicit the progesterone-dependent reproductive responses necessary for female fertility, while PR-B is required to elicit normal proliferative responses of the mammary gland to progesterone [3].

Progesterone and estrogen are secreted by the ovary, as well as the placenta of pregnant animals, and these hormones are mainly involved in the growth and development of the mammary gland during puberty and pregnancy [4, 13, 17, 18]. These hormones have additional roles during lactogenesis and lactation. The dynamic regulation of the progesterone receptor plays significant role in successful pregnancy [2, 8, 9, 16, 20] signaling initiated by PGR within the uterine microenvironment during implantation period promotes implantation of conceptus and also promotes the development and maintenance of gestation [1, 19]. Peiró et al. (2008) found the single nucleotide polymorphism in rabbit PGR promoter and the association between different litter size, implanted embryos, and early embryo survival.

II. DETAILS EXPERIMENTAL

2.1. Material and Methods

The trial was performed on the experimental rabbit farm at the National Agricultural and Food Centre, Nitra, Slovak Republic. A total number of 204 clinically healthy adult animals (179 does and 25 bucks) of local crossbreed line of New Zealand white x Californian x rabbit of Nitra were used.

Animals were individually housed in wire cages arranged in flat-decks on one level. The rabbits were fed with a commercial diet. All animals were given access to the feed ad libitum. Drinking water was provided with nipple drinkers ad libitum. A cycle of 16 h of light and 8 h of dark was used throughout the trial. Temperature and humidity in the building were recorded continuously by a digital thermograph. Heating and forced ventilation systems allowed the building temperature to be maintained within 18±4°C throughout the trial. Relative humidity was in interval 70 ± 5%.

In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by ethical committee.

2.2. Evaluated reproduction traits

All females were at least after the third kindling and were artificially inseminated (A.I.) by fresh heterosperm semen doses (0.5 ml per one female). Each female was administered intramuscularly 2.5 µg

synthetic GnRH-Lecirelinum immediately after A.I.
Number of total born kits, live born kits, stillborn kits per litter and pre-weaning mortality were observed. The milk production was calculated by formula [5].
Milk yield 0-21 d (g) = 1.69 x weight gain of the litter 0-21 d (g) + 362

2.3. Molecular analyses

Peripheral blood for DNA isolation was collected from vena auricularis centralis into EDTA sample tubes. For automated isolation and purification of DNA was used MagNA Pure LC 2.0 Instrument and MagNA Pure LC DNA Isolation Kit I (Roche, USA) following the manufacturer's instructions. Total DNA concentrations were measured by UV/VIS spectrophotometer NanoPhotometer (Implen).
For the amplification of PGR promoter fragment (558-bp) and detection of polymorphisms in this segment were designed and synthesized specific primers according to [11] and using gene sequence (GenBank, X06623):

PGR F: 5́-GAAGCAGGTCATGTCGATTG GAG-3´
PGR R: 5́-CGCCTCTGGTGCCAAGTCTC-3´
The PCR conditions (PTC-200, BIO-RAD) were 95°C for 2 minutes, 94°C for 30 s, 66°C for 30 s, 72°C for 30 s, 35 cycles, with last extension at 72°C for 10 min. The reaction volume (25µl) contained 10 mM Tris-HCl (pH 8.6 at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$, 25 units/ml Taq DNA polymerase, 0.2 mM dNTPs each, 5% glycerol, 0.08% IGEPAL® CA-630, 0.05% Tween-20) (New England Biolabs), 10 pmol/µl each primers.

Genotyping

PCR-RFLP method with restriction enzyme Eco31I was used for PGR genotyping.
PGR amplicon was digested by 5 IU of Eco31I (Fermentas) 37°C/16 hrs. The restriction fragments of three different PGR genotypes (AA 558bp, GG 416+142 bp and AG 558+416+142 bp) obtained in digest reactions were electrophoretically separated on 2% agarose gels containing ethidium bromide at 80 mA in 10mM lithium borate buffer, pH 8.0 for 60 minutes. The products were visualized under UV light and photographed using a MiniBis Pro (Bio-Imaging Systems).

2.4. Statistical analyses

Statistical analysis of the obtained parameters and allele frequency were statistically evaluated by chi-squared test, one-way analysis of variance (ANOVA) with Scheffe´s test and t-test with the level of significance set at p values of less than 0.05, 0.01 and 0.001. The results are quoted as means ± standard deviation.

III. RESULTS AND DISCUSSION

The polymorphism and three different genotypes (AA, AG and GG) in PGR gene promoter were detected by used PCR RFLP method (Fig. 1). The highest frequency reached of genotype AG (0.44), then genotype GG (0.33) and the lowest frequency was in genotype AA (0.24). The G allele frequency was 0.54 and the A allele frequency was 0.46.

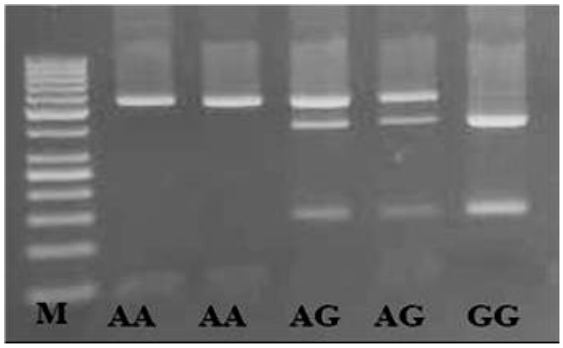

Fig.1. PCR-RFLP for PGR gene (genotypes AA 558bp, GG 416+142 bp and AG 558+416+142 bp)

When comparing the observed and expected genotyping frequencies with the $\chi 2$ test, the results were statistically inconclusive and the tested rabbit population was in equilibrium.

Parameter	Female genotype					
	AA (n=42)		AG (n=78)		GG (n=59)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Liveborn kits/litter (n)	8.52^{ab}	3.37	8.45^{a}	2.86	9.71^{b}	2.34
Milk production (g)	4032.6^{a}	414.78	4175.32^{b}	380.1	4871.4^{c}	433.93
Pre-weaning mortality (%)	16.22^{a}	-	35.64^{b}	-	13.44^{a}	-

Table 1: Productive and reproductive traits in different rabbit PGR genotypes

Means ±SD with different superscripts in the same row significantly differ at $p < 0.05$ (a, b); $p < 0.001$ (a,b,c).

In the group of does with GG genotype we observed significantly higher ($p<0.05$) total born kits compared to genotype AG as well as the significantly higher numbers of live-born kits were found also in genotype GG (Table 1). However we did not find statistically significant differences between genotypes in the evaluation of still-born kits.

Peiró et al. (2008) observed 589 females selected for uterine size for ten generations. They monitored PGR as a possible candidate gene affecting litter differences and related factors (embryo number and survivability, developmental stage). In their work they focused on SNP analysis at 2464 G → A. Allele G was identified in 75% of animals with large uterine capacity and only 25% of animals with less uterine

capacity. At the same time, the GG genotype animals were 0.5 progeny more litter than the AA genotype animals and obtained the same results when observing the number of implanted embryos. In the 48 hours of pregnancy they found negligible differences in number and degree of embryo development were, however at 72 hours the GG genotype had by 0.36 embryos more than AA genotype. Also our results confirm better reproductive properties GG genotype (9.71 liveborn kits/litter) compared with AA and AG. The females of genotype GG had highest milk production (4871.4 g/doe) during first 21 days (Table 1). In contrary the lowest milk production was recorded in genotype AA, however the highest pre-weaning mortality ($p<0.001$) was once again in genotype AG compared wit other two genotypes. The genotype AG was not evaluated by Peiró et al. (2008).

IV. CONCLUSION

We found a higher frequency of heterozygous genotype AG compare AA and GG. The smallest proportion was the mutated AA genotype. The frequency of G allele is slightly higher than A allele. $\chi 2$ test evaluation of differences between the theoretical and observed genotype counts, we calculated that the population is in genetic balance.

The association studies focused on the effect of AA, GG and AG genotype on liveborn kits per litter confirmed significant differences. The females of GG genotype reached the highest litter size followed by AA female. The lowest values of litter size were found in group of AG genotype. There were no differences between genotypes in average number of stillborn kits per litter. The females of genotype GG had highest milk production.

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