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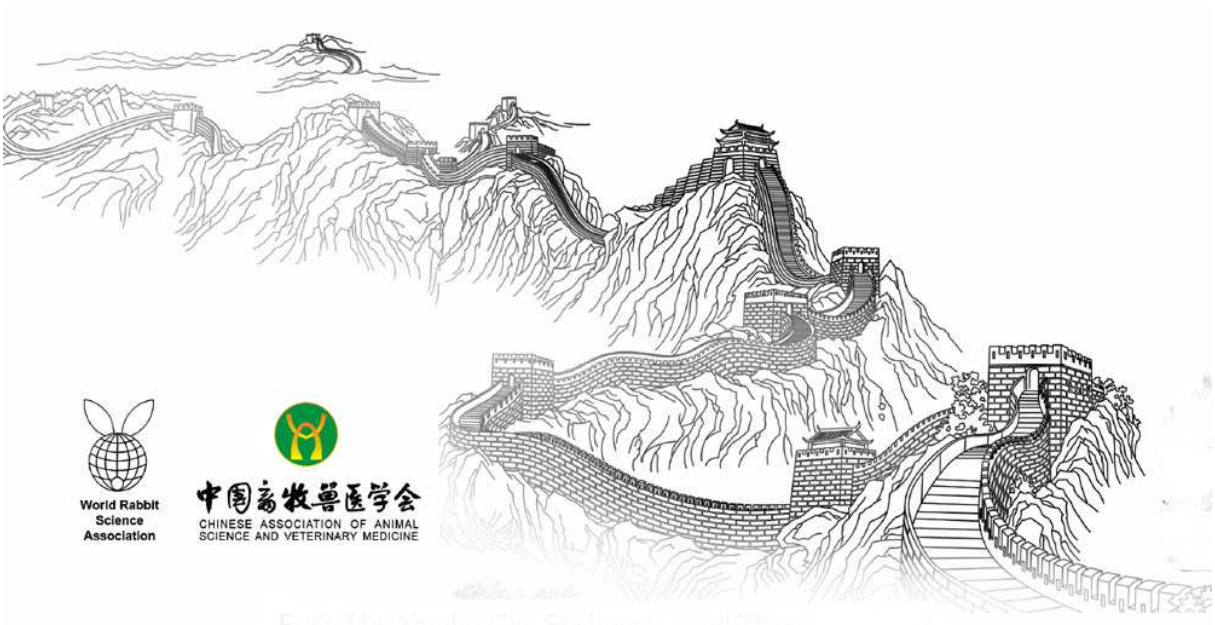

PHENOTYPIC VARIATION OF TEAT NUMBER IN CHUANBAI REX RABBIT AND ASSOCIATION WITH SNPS POLYMORPHISM OF ESR AND FSHβ GENES

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ABSTRACT

Teat number is an important trait in relation to reproduction performance in farm animals, which, however, has not been carefully studied in Rex rabbit yet. In the present study, we first investigated the teat number among 293 Chuanbai Rex rabbit and found that there was adequate phenotypic variation with the predominant type of 8 teats (54.9%). No other type with lower than 8 teats or higher than 10 teats was observed. We also supported the significant associations between teat number and main reproduction traits; however, these associations would be changeable among different parities. We subsequently scanned the SNPs polymorphism for *ESR* and *FSHβ* genes by direct sequencing approach and further revealed that there was no significant association between the genotypes and phenotypes of teat number. Our results confidentially supported the possibility that the trait of teat number in Chuanbai Rex rabbit can be improved through the artificial selection, which should be included into the future breeding program.

Keywords: Rex rabbit, Teat number, SNPs polymorphism, *ESR* and *FSHβ* genes, Association analysis

INTRODUCTION

Rex rabbit is an European breed and famous for the production of short dense plush velvet-like fur. In China, the breeding of Rex rabbit is very prevalent with total number of 50 million rabbits in stock in 2014, which annually produced 30 million pieces of fur (Liu *et al.*, 2014). Based on these initially imported Rex populations, a few of cultivated rabbit breeds have been developed and widely distributed, such as the Chuanbai Rex rabbit. The teat number is an important phenotype in relation to reproduction performances in farm animal, which has been characterized by moderate heritability in pig (Xu *et al.*, 2013). Therefore, many candidate function genes, such as the estrogen receptor (*ESR*) and follicle stimulating hormone β (*FSHβ*), have been reported to be associated with the phenotype of teat number in pig (Li *et al.*, 2007). Meanwhile, many QTLs were also proposed to determine the teat number in pig (Ding *et al.*, 2009; Hu *et al.*, 2013). In contrast to pig, the phenotypic variations of teat number and potential candidate genes have not been investigated in rabbit yet.

In the present study, we provided the primary results on this subject in Chuanbai Rex rabbit, which would be useful for guiding the future genetic selection and improvement of teat number in rabbit.

MATERIALS AND METHODS

Animals and investigation of teat number

A total of 293 adult females of Chuanbai Rex rabbit were randomly selected from commercial farms in Sichuan for investigating the teat number by visual inspection. During this process, more attentions should be paid to avoid potential mistakes. For these rabbits, we further recorded their traits in relation to

reproduction performances, including the total born (TB), the live born (LB), birth litter weight (BLW), and 21d litter weight (DLW) for parities 1-3rd.

Gene sequencing and SNPs scanning

Eight pairs of primers were newly designed to amplify the nine exons (exons 1 - 9) of rabbit *ESR* gene. Meanwhile, two pairs of primers were also used to amplify the three exons (exon1 - 3) of rabbit *FSHβ* gene. Subsequently, we directly sequenced the PCR amplification products for scanning SNPs among 92 samples, which could be classified into three types according to the phenotype of teat numbers (8 teats, N = 36; 9 teats, N = 27; 10 teats, N = 29).

The proposals of PCR amplification was performed at 94 °C for 3 min, and then subjected to 30 cycles at 94 °C , for 30 s, 56 °C for 30 s, and 72 °C for 1 min 30 s, followed by a final extension at 72 °C for 5 min, and was finally terminated at 4 °C. The PCR products were separated by electrophoresis on a 2% agarose gel and sequencing by ABI Prism® 3730 instrument (U S).

Data analysis

After the investigation of phenotypic variation of teat number in commercial population of Chuanbai Rex rabbit, we conducted the one way ANOVA between teat number and reproduction traits of interest with General Linear Model (GLM) procedures of SAS V8.22,. Furthermore, we also analyzed the genetic variation of ESR and FSHβ genes and their associations of different genotypes with teat numbers in this rabbit population by Chi-square tests procedures of SAS V8.22.

RESULTS AND DISCUSSION

We directly counted the teat number among 293 Chuanbai Rex rabbit and totally found three types, including the types of 8 teats (N = 161, 54.9%), 9 teats (N = 86, 29.4%), and 10 teats (N = 46, 15.7%). Actually, previous study already reported that the teat number in pig showed the normal distribution in statistic with mean value of 12 teats (Pi *et al.*, 2010). Therefore, the patterns of statistical distribution for teat number are different between rabbit and pig, which would be due to the species-specific laws. Furthermore, our results in the present study first provided the evidences that there was significant variation of teat number in Rabbit. Additionally, no other type of teat number was observed in Chuanbai Rex rabbit.

Table 1: Association analysis between teat number and four reproduction traits (Total number born: TB; number born alive: LB; birth litter weight: BLW and 21d litter weight: DLW)

Teat number	First parity	Second parity	Third parity
TB			
8	7.16 ± 1.22 (161)	7.76 ± 1.45 (136)	8.56 ± 1.52 ^{a} (102)
9	7.05 ± 1.56 (86)	7.69 ± 1.21 (68)	7.66 ± 1.20 ^{b} (56)
10	6.82 ± 1.42 (46)	7.95 ± 1.37 (38)	7.68 ± 1.07 ^{b} (25)
LB			
8	7.09 ± 1.12 (159)	7.46 ± 1.32 (134)	8.20 ± 1.35 ^{a} (98)
9	6.88 ± 1.51 (86)	7.48 ± 1.02 (67)	7.48 ± 1.10 ^{b} (56)
10	6.64 ± 1.42 (45)	7.75 ± 1.27 (36)	7.36 ± 1.39 ^{b} (28)
BLW(g)			
8	373.20 ± 64.78 ^{a} (148)	417.76 ± 76.46 (133)	451.73 ± 70.66 (94)
9	353.54 ± 61.94 ^{ab} (80)	405.24 ± 70.32 (70)	425.47 ± 66.27 (55)
10	338.11 ± 71.23 ^{b} (46)	432.97 ± 94.78 (39)	427.73 ± 94.12 (26)
DLW(g)			
8	2050.21 ± 333.33 (115)	2242.75 ± 349.27 (110)	2249.34 ± 435.24 (76)
9	1990.19 ± 351.66 (59)	2245.43 ± 395.98 (60)	2152.71 ± 459.42 (49)
10	1897.82 ± 394.01 (34)	2162.00 ± 309.85 (31)	2030.40 ± 421.81 (20)

Note: The number in brackets represents statistical sample number; significant differences between distinct types of teat number were denoted by the upper letters ($p < 0.05$).

The association analysis between teat number and reproduction traits are shown in Table 1. On the whole, we detected the significant association between teat number and TB, LB, and BLW. However, their association could be varied among different parities. This result would cast more attentions when they are employed into practical application. In details, the TB and LB from rabbits of 8 teats were significantly higher than from individuals of 9 teats and 10 teats on the third parity ($p < 0.05$). The BLW trait on the first parity was detected to be the highest record in rabbits of 8 teats, which was significant higher than that from individuals of 10 teats. Finally, we didn't find the significant differences of DLW traits among three types of teat number. In contrast to rabbit, there are various reports supporting the significant association between teat number and reproduction traits in pig (Gao *et al.*, 2011), which also facilitates the intention that the teat number should be considered into breeding program for improving selection progress of reproduction performance. However, we still lack the obvious evidences in rabbit for supporting the importance of teat number in relation to reproduction performances.

By direct sequencing, we only detected one SNP (c.195A>G) in exon 3 of *ESR* gene and one SNP (u.187C>G) in 3' UTR region of *FSHβ* gene. The frequencies of genotypes for c.195A>G SNP were 30.4% (AA), 47.8% (AG), and 21.7% (GG), respectively, which was under the Hardy-Weinberg Equilibrium (HWE). However, the frequency distribution of u.187C>G SNP was in deviation from HWE with 19.6% CC, 25.0% CG, and 55.4% GG genotypes. Both *ESR* and *FSHβ* genes have been widely proposed to be involved into reproduction traits and significantly associated with teat number in pig (Li *et al.*, 2007). However, our result showed that there were no significant association (Chi-square tests，$P > 0.05$) between genetic polymorphism of both *ESR* and *FSHβ* genes and phenotype of teat number. Of course, we can't absolutely exclude the potential false negative results because only 92 rabbits were genotyped in the present study and subjected to association analysis.

CONCLUSIONS

Our result confidentially revealed that there is an adequate phenotypic variation of teat number trait in Chuanbai Rex rabbit, which supported the possibility for genetically selecting and improving the teat number trait. To our knowledge, this was the first report for investigating phenotypic variation of teat number and association with reproduction traits in Rex rabbit.

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