



Short Communication

Immunisation of Somatostatin DNA Vaccine in Ewes Can Affect Serum Somatostatin and Prolactin Levels in Offspring Lambs Through Maternal Related Hormones

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ABSTRACT

This study aimed to analysis the effect of immunisation of somatostatin (SS) DNA vaccine in maternal animals on serum related hormones in offspring. In this study, ten ewes with two newborn lambs were randomly divided into SS DNA vaccine group (Group T) and empty vector group (Group C), and these vaccines were given orally into the ewes at weeks 0, 3 and 6 of the research. Blood samples of ewes or offspring lambs were collected at week 6 or 9 of lactation, respectively. Serum SS, prolactin (PRL), growth hormone (GH) and insulin-like growth factor 1 (IGF-1) concentrations were detected by radioimmunoassay. The offspring lambs in T group showed significantly lower serum SS and higher PRL concentrations than those in the control group, and there were no difference between T group and the control group in terms of serum GH and IGF-1 concentrations. There was a significant negative correlation between offspring lambs serum SS and GH, IGF-1, PRL concentration, and there was a significant positive correlation between offspring lambs serum PRL and GH, IGF-1 concentration. There was a significant positive correlation between ewes serum SS concentration and offspring lambs serum SS concentration, ewes serum PRL concentration and offspring lambs serum PRL concentration. The findings indicate that the immunisation with oral somatostatin DNA vaccine in ewes may affect serum SS and PRL levels in offspring lambs through maternal related hormones.

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Authors' Contribution

YGH and YFH planned the experiment. YGH, JYW and YQH executed the experiment and drafted the manuscript. RSN, JG, YZ, GXE, YJZ and YFH helped in laboratory work, statistical analysis and preparation of manuscript.

Key words

Somatostatin, DNA vaccine, Hormones, Offspring lambs, Ewes

Somatostatin (SS) can inhibit animal lactation through the action of growth hormone (GH) and prolactin (PRL). SS mainly derived from the hypothalamus can suppress GH-IGF-1 (insulin-like growth factor 1) axis and secretion of PRL in the anterior pituitary (Yang *et al.*, 2017; Luque and Kineman, 2018; Han *et al.*, 2019). GH plays an important role in promoting mammary gland growth, development, milk synthesis and secretion (Lékó *et al.*, 2017; de Moraes *et al.*, 2017). Many studies have found that giving GH can increase milk production of animals and GH antibody treatment or absence of GH can significantly reduce milk production (Akers, 2017; Lékó *et al.*, 2017; Cui *et al.*, 2018). GH mainly derived from the pituitary can stimulate the synthesis and secretion of IGF-1 in the liver, mammary gland and other peripheral tissues (Bao *et al.*, 2016; Dosouto *et al.*, 2019).

IGF-1 is the most important mediator of the actions of GH, and it can effectively stimulate mammary gland growth, development and lactation in the GH axis (Villa-Osaba *et al.*, 2016; Hoeflich and Meyer, 2017; Lékó *et al.*, 2017). PRL also plays a vital role in maintaining mammary gland development and lactation in animals (Lacasse *et al.*, 2016, 2019; Saleem *et al.*, 2018). The absence of PRL can cause approximately 50% reduction in rat milk yield (Maddon *et al.*, 1986). Therefore, SS can affect the secretion of GH, IGF-1 and PRL during animal lactation.

Immunisation of SS DNA vaccine is an effective way in promoting animal lactation through the action of GH-IGF-1 axis and PRL. The active immunisation of exogenous SS can effectively neutralize endogenous SS (Dong *et al.*, 2018; Han *et al.*, 2019) and promote weight gain of offspring during lactation by improving the secretion of the mother GH, PRL and IGF-1 (Bai *et al.*, 2011; Dong *et al.*, 2018). Therefore, immunisation of SS DNA vaccine can affect the levels of serum SS, GH, IGF-1 and PRL in maternal animals. However, it is still unclear about the

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affect of immunisation of SS DNA vaccine on serum related hormones such as SS, GH, IGF-1 and PRL in offspring, and the relationship between lamb and ewes serum related hormones after immunisation of SS DNA vaccine.

In this study, we studied the affect of immunisation of SS DNA vaccine in maternal animals on serum related hormones such as SS, GH, IGF-1 and PRL in offspring animals, and the relationship between offspring animals serum related hormones and maternal related hormones.

Materials and methods

After parturition, ten healthy female Dazu Black goats with two new born offspring lambs per ewe were purchased and fed in Ruifeng Modern Agriculture Development Co., Ltd of Chongqing Dazu District in China. All goats were fed according to the guidelines of the Committee on the Care and Use of Laboratory Animals of China, and all animal experiments were permitted by the Science and Technology Ethics Committee of Southwest University.

Ten healthy Dazu Black goat ewes with two new born offspring lambs per ewe were randomly divided into the SS DNA vaccine group (Group T) and control group (Group C). These ewes were firstly given orally 10 mL of 7.5% sodium bicarbonate solution at 30 min before immunisation, then separately given orally C500 (ptCS/2SS-asd) vaccine (Group T) and C500 (pVAX-asd) empty plasmid (Group C) at a dose of 5×10^{11} CFU dissolved in 5 mL sterile saline solution. These ewes were immunised total three times with a three-week interval. Ewes serum were collected at week 6 of lactation, and offspring lambs serum were collected at week 9 of lactation by centrifugation $1157 \times g$ for 10 min and stored at -20°C until use.

Ewes and offspring lambs serum SS, GH, IGF-1 and PRL concentrations were detected by radioimmunoassay kit (Beijing Sino-UK Institute of Biological Technology, Beijing, China). The assay sensitivities for SS, GH, IGF-1, PRL was < 1.0 pg/mL, < 0.1 ng/mL, < 10 ng/mL and < 12.5 uIU/mL, respectively. The intra-assay coefficients of variation for SS, GH, IGF-1, PRL assays were $< 9.5\%$, and the inter-assay coefficients of variation for SS, GH, IGF-1 and PRL assays were $< 5\%$.

Statistically significant differences ($P < 0.05$) between vaccine group and control group in terms of ewes and offspring lambs serum SS, GH, IGF-1 and PRL were analysed by one-way ANOVA of SAS 8.1 (SAS Institute, Inc., Cary, NC, USA). All data were expressed as mean \pm SD.

Results and discussion

The offspring lambs in vaccine group showed significantly lower serum SS concentration than those

in control group at week 9 of lactation (Fig. 1A, $P < 0.05$), and the offspring lambs in vaccine group showed significantly higher serum PRL concentration than those in control group at week 9 of lactation (Fig. 1D, $P < 0.05$). However, no significant difference was observed between vaccine and control group in terms of offspring lambs serum GH and IGF-1 concentration at week 9 of lactation (Fig. 1B, C, $P > 0.05$).

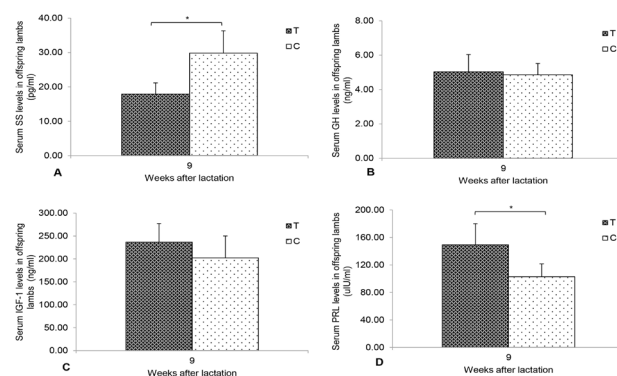


Fig. 1. Changes in serum related hormones levels in offspring lambs after immunisation of SS DNA vaccine. A, SS concentration (pg/ml) in T and C groups at week 9 of lactation; B, GH concentration (ng/ml) in T and C groups at week 9 of lactation; C, IGF-1 concentration (ng/ml) in T and C groups at week 9 of lactation; D, PRL concentration (uIU/ml) in T and C groups at week 9 of lactation, Data are shown as means \pm SD. * $P < 0.05$.

There was a significant negative correlation between offspring lambs serum SS and GH, IGF-1, PRL concentration (Table I, $P < 0.05$). There was a significant positive correlation between offspring lambs serum PRL and GH, IGF-1 concentration, and between offspring lambs serum GH and IGF-1 concentration (Table I, $P < 0.05$).

Table I. Correlation coefficients among serum SS, GH, IGF-1 and PRL in offspring lambs at week 9 after lactation.

	SS	GH	IGF-1	PRL
SS	1			
GH	-.532*	1		
IGF-1	-.807*	.767*	1	
PRL	-.850*	.567*	.828*	1

* $P < 0.05$ indicates significant difference between each other.

There was a significant positive correlation between ewes serum SS concentration and offspring lambs serum SS concentration (Table II, $P < 0.05$), ewes serum

PRL concentration and offspring lambs serum PRL concentration (Table II, $P < 0.05$). There was a significant negative correlation between offspring lambs serum SS and ewes serum PRL concentration (Table II, $P < 0.05$).

Table II. Correlation coefficients among serum SS, GH, IGF-1 and PRL in ewes and offspring lambs.

	SS in lamb serum	GH in lamb serum	IGF-1 in lamb serum	PRL in lamb serum
SS in ewes serum	0.909*			
GH in ewes serum	-0.389	-0.121		
IGF-1 in ewes serum	-0.450	0.216	0.414	
PRL in ewes serum	-0.722*	0.257	0.492	0.727*

* $P < 0.05$ indicates significant difference between each other.

SS, GH, IGF-1 and PRL play a vital role in the regulation of somatostatin DNA vaccine on animal lactation and the weight gain of offspring during lactation. SS plays an important role in the regulation of animal lactation by the GH-IGF-1 axis and PRL (Bai *et al.*, 2011; Luque and Kineman, 2018; Dong *et al.*, 2018). Many previous studies found that immunisation of SS DNA vaccine can induce the production of specific SS antibodies in animals (Dong *et al.*, 2018; Han *et al.*, 2019), which can effectively neutralize endogenous SS, then promote the secretion of GH-IGF-1 axis and PRL, and ultimately promote the weight gain of offspring during lactation (Bai *et al.*, 2011; Dong *et al.*, 2018). In addition, SS also plays a vital role in the regulation of animal growth by the GH-IGF-1 axis (Murray and Clayton, 2013) and immunisation of SS DNA vaccine can promote the growth of animals (Xue *et al.*, 2010; Liang *et al.*, 2014). In addition to improving milk yield and quality by GH-IGF-1 axis and PRL to promote the growth of offspring (Bai *et al.*, 2011; Dong *et al.*, 2018), immunisation of SS DNA vaccine in mothers may also affect the growth of offspring through maternal related hormones such as SS, GH and IGF-1. Therefore, it will enable us to more fully understand the mechanism of somatostatin gene immunisation in promoting the growth of offspring through studying the affects of immunisation of SS DNA vaccine on serum SS, GH, IGF-1 and PRL in offspring animals, and the affects of maternal-related hormones on growth-related hormones in offspring after immunisation of SS DNA vaccine.

In this study, immunisation of SS DNA vaccine in ewes can affect serum SS and PRL levels in offspring lambs. The offspring lambs in vaccine group showed significantly lower serum SS and higher PRL concentrations than those in the control group, and there

were no difference between the vaccine group and control group in terms of serum GH and IGF-1 concentration. Bai *et al.*'s study found that immunisation of SS DNA vaccine in mother mice did not affect serum SS antibody concentration and GH concentration in offspring mice (Bai *et al.*, 2011). Our results indicated that immunisation of SS DNA vaccine in mother animals can affect serum SS and PRL levels in offspring, which is not caused by the serum SS antibody in offspring neutralizing the serum endogenous SS. Thyroid hormone can promote the growth and development of animals in infancy and SS can inhibit the secretion of thyroid hormone (Behringer *et al.*, 2018; Rodríguez-Álvarez *et al.*, 2015). Therefore, lower serum SS in offspring lambs may promote the growth of lambs through improving the secretion of thyroid hormones. PRL can promote liver growth of animals (Moreno-Carranza *et al.*, 2018) and therefore higher serum PRL in offspring lambs may promote the growth of lambs through the liver function.

In the research, maternal serum related hormones may affect the serum related hormones levels of offspring after immunisation of SS DNA vaccine in ewes. There was a significant positive correlation between ewes serum SS concentration and offspring lambs serum SS, ewes serum PRL concentration and offspring lambs serum PRL concentration, and there was a significant negative correlation between offspring lambs serum SS and ewes serum PRL concentration. In addition, there was a significant negative correlation between offspring lambs serum SS and GH, IGF-1 and PRL concentration, and there was a significant positive correlation between offspring lambs serum PRL and GH, IGF-1 concentration, and there was a significant positive correlation between offspring lambs serum GH and IGF-1 concentration. Previous studies also found that there was a significant negative correlation between maternal serum SS and GH, IGF-1 concentration, and there was a significant positive correlation between maternal serum GH and IGF-1 concentration after immunisation of SS DNA vaccine (Xue *et al.*, 2010; Han *et al.*, 2014). These results indicated that offspring serum SS and PRL concentrations can be affected by maternal serum SS and PRL after immunisation of SS DNA vaccine in maternal animal. This affects may be caused by sucking milk of the offspring which is due to large amount of SS and PRL in the milk (Healy *et al.*, 1980; Holst *et al.*, 1990).

Conclusion

The immunisation with oral somatostatin DNA vaccine in ewes may affect serum SS and PRL levels in offspring lambs through maternal hormones. Further studies will focus on the mechanism of maternal hormones affecting offspring hormonal changes after immunisation of SS DNA vaccine.

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Statement of conflict of interest

Authors have no conflict of interest to declare.

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