THE EFFECTS OF LRH-A_2 AND RARE-EARTH ON TESTICULAR FUNCTIONS OF ANGLA MALE RABBITS SUFFERING OF HEAT STRESS

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Abstract - In the experiment, 32 Tang Hang Angla male rabbits [Angora strain] suffering of heat stress were randomly divided into 4 groups of 8 rabbits. Each rabbit in the first group was inoculated intramuscularly with 5μg of LRH-A_2 once every 3 days (5 times), then once every 5 days(3 times) covering a period of one month. Thus, each rabbit of the group 1 was inoculated with 5μg of LRH-A_2 for 8 times during this experiment. Rabbit of the second group were fed with the feed mixed with 200ppm of rare-earth. Rabbits of the third group were both inoculated with LRH-A_2 for the same amount as that in the first group and fed with the feed mixed with the same amount of rare -earth like that in the second group. The fourth group (the control group) was not treated with any of the methods used for the other three groups. The feed for each rabbit in the four groups was distributed in the same amount during the 30 days experiment, *i.e.*150g of a concentrate in form of meal (consisting of DE 2100 kcal/kg, CP 16.2%, CF 28.5%) and 400g of green grass every day. The following items of each rabbit were tested at both the beginning and at the end of the experiment: measuring testicular size; volume of ejaculate; sperm density and vigour. Meanwhile, 30 female rabbits were inseminated with sperm of each group randomly and the pregnancy rates were also recorded. The results showed the rabbits in the three experimental groups respectively changed greatly in the following items compared with those at the beginning of the experiment. 1/ testicular size increased by 9.94%, 5.37% and 16.71% ($P>0.05$) while those in the control group decreased by 26.56% ($P<0.05$). 2/ The volume of ejaculate increased by 0.28, 0.31, 0.24 and 0.13ml. 3/ The density of spermatozoa increased by 0.1, 0.07, 0.65 and 0.1 hundred million/millilitre. 4/ The vigour of the spermatozoa increased by 0.23, 0.24, 0.42 and 0.09. 5/ The average numbers of the pregnant female rabbits were 19, 20, 23, and 11. 6/ The average pregnancy rates were 63.33%, 66.67%, 76.67% and 36.62%. The above results showed that the best result were in group 3 ($P<0.01$or $P<0.05$). This preliminary experiment showed LRH-A_2 or rare-earth had positive effects on male rabbits suffering of heat stress for restoration of their testicular function. It was believed that much better effects could be obtained if the above two elements are applied simultaneously to rabbits.

INTRODUCTION

It was proved that the epithelium of testicle producing sperm would decline and lose it's function of sperm making in the condition above 30°C since male rabbits are greatly sensitive to high temperature. The best example was the phenomenon of "no pregnancy in summer" in the southern part of China. The high temperature also lowered variously the reproduction rates in the northern part of China. The conception rates and the number of parturitions were greatly reduced till the middle of Autumn. This had caused a big problem in the reproduction of the Angla Rabbits. For the purposes of reducing the damage of high temperature to male rabbits' testes, shortening the recovering time of the testes and increasing the breeding rates of the rabbits, the experiment was made.

MATERIAL AND METHODS

Material

Rabbits - 32 adult Tang Hang Angla Male Rabbits [Angora strain] suffering of heat stress were elected and one by one their testicular size and the quality of their seminal fluid were tested before they were randomly divided into 4 groups in the East Rabbit Farm of Gao Yi County.

LRH-A_2 - made in Ning Bo Hormone Products Factory, was also named "ovulation promoting-2". Its batch number was 910202, an concentration 2μg per ampoule.

Rare-earth - made in Nei Monggol Rare-Earth Smeltery, was a kind a pink powder of rare-earth oxide consisting of the following major components: $Ce(NO_3)_3$,38.75% - $La(NO_3)_3$, 39.25% - $Pr(NO_3)_3$,5.30% - $Nd(NO_3)_3$, 15.50% and $Sm(NO_3)_3$, 1.18%.

Methods

Treatment of the experimental rabbits - The 32 rabbits were randomly divided into four groups and each group consisted of 8 rabbits. Each rabbit in the first group was inoculated intramuscularly with 5μg of LRH-A_2 once every 3 days(5 times), then, 3 times every 5 days (3 times). So, each rabbit was inoculated for 8 times during the experiment. Those in the second group were only fed with the feed mixed with 200 ppm of rare-earth. The rabbits in group three were both inoculated with the same amount of LRH -A_2 as that in group one and fed with the same amount of rare-earth like in group two. The rabbits in the fourth group were untreated with the above methods. It was the control group.

Raising and management - The experiment was made from 5 September to 5 October 1991 in the East Rabbit Farm of Gao Yi County. Each rabbit in different groups was raised in a single cage covering an area of 0.25m^2 with good sunlight and ventilation. Each rabbit was fed two times every day with 150g of a compound meal feed (added of some water). The feed contained wheat bran 42%, peanut shells 42% and peanut cake 16%. The calculated major nutrients of the feed were DE 2100 kcal/kg, CP 16.20%, CF 28.52 %. In addition to that, each rabbit was fed with 400g of green grass and enough amount of water. All the rabbits were respectively cut wool and inoculated immune vaccine once and were looked after by a particular person during the experiment. It was needed to state that the conditions were the same for the rabbits in the four groups.

Testicle Size Measuring - The size of the rabbit male testes was measured with vernier calliper separately (testicle length x width x thickness) at both the beginning (5 September 1991) and at the end (5 October 1991) of the experiment (DU YUCHUAN *et. al.*, 1987). In the process of measuring, the male rabbit was held by an assistant while the operator pushed the rabbit's testes into its scrotums and kept them at the end of the scrotum with two fingers in the left hand, keeping the testes in their basic form.

Ejaculate volume and fresh semen characteristics - The seminal fluid of each rabbit was separately collected at the beginning and at the end of the experiment. Then, the sperm volume, colour, density as well as the number and motility of the spermatozoa were all recorded. The method used to measure the spermatozoa density was using a blood-cell counting board, which was in use for other domestic animals. The motility was observed with a microscope magnifying 400 times at the condition of 30-35°C, watching the sperm's moving rate following a straight line.

Other parameters - 360 During the experiment, the spirit, the appetite and the diseases of the rabbits were carefully observed. The rabbits' sexual desire was also observed when their seminal fluid was collected..

RESULTS

Testicular size (Table 1)

During the experiment, the testicular size of the male rabbits increased in the three experimental groups, particularly for group three. The average increase in group three was 16. 71%(P>0. 05) while the control group decreased by 26.57% (P <0.05).

Table 1 : Changes of the testicular size of the male rabbits.

Unit cm^3, %

Time	Beginning			End			Changing	
Size Groups	Left	Right	Average	Left	Right	Average	cm^3	%
1	3.59	3.05	3.32	3.87	3.42	3.65	+0.33	+9.94
2	4.51	4.43	4.47	4.78	4.64	4.71	+0.24	+5.37
3	3.72	3.46	3.59	4.30	4.08	4.19	+0.60	+16.71
Control	4.65	3.93	4.29*	2.96	3.33	3.15	-1.14	-26.57

* P<0.05

Ejaculate volume and fresh semen characteristics

From Table 2, it could be seen that the ejaculate volume and fresh semen characteristics of male rabbits in the three experimental groups had been sharply improved ($P<0.05$), particularly for spermatozoa density and motility. Although these were also improvement to some extend in the control group, the difference was not great compared with those at the beginning of the experiment.

The insemination results showed the fertility rates in the three experimental groups were all above 60%, particularly in group three reaching by 76.67% while this was only 36.67% in the control group The differences among the three experimental groups was not great ($P>0.05$) while the difference between the control group and the experimental groups one and two was very sharp ($P<0.05$), and the difference between the control group and experimental group three was much more sharp ($P<0.01$).

Table 2 : The ejaculate volume and fresh semen characteristics

Unit : ml, hundred million/ml (vol.) (sperm density)

Time	Beginning			End			Changing			Insemination		
Size	Vol (ml)	sperm density	motility rate	vol (ml)	sperm density	motility rate	vol (ml)	spergnant density	motility rate	females (N)	pregnant	pregnancy rate
1	0.38	0.90	0.13	0.66*	1.00	0.36*	+0.28	+0.1	+0.23	30	19	63.3^{ab}
2	0.52	0.93	0.19	0.83*	1.00	0.43*	+0.31	+0.07	+0.24	30	20	66.67^{ab}
3	0.53	0.80	0.18	0.77*	1.45*	0.60**	+0.24	+0.65	+0.42	30	23	76.67^{a}
control	0.43	0.80	0.25	0.56	0.90	0.34	+0.13	+0.10	+0.09	30	11	36.67^{c}

* $P<0.05$, ** $P<0.01$

Other parameters

During the experiment, the spirit and appetite of the rabbits in all groups were very normal because there were not any diseases in this period while the sexual desire of the male rabbits in the experimental groups increased also. At the beginning there were two rabbits with no spermatozoa in experimental group one and three and one rabbit with dead spermatozoa in group two. After they were treated, they had not only alive spermatozoa, but the spermatozoa vigour was also over 0.2.

DISCUSSION AND CONCLUSION

LRH-A_2 is a kind of composed matter similar to the hormone of nine peptides but lack of one AA than the natural one. It's activity is several times higher than the natural GnRH though it molecular weight is 1166. LRH-A_2 could make the animals hypophysis secreting LH and FSH but its function was mostly secreting LH. It is mainly used in the pressing sexual passion promoting ovulation, exceeding ovulation and synchronisation of oestrus for the domestic animals.

The LH secreted by the front part of the hypophysis may promote the between-cells of testicle secreting testicular ketone, which may promote the division of the reproducing cells and the forming of the spermatozoa. Moreover, it could control the developing and secretion of the annex sex glands. FSH greatly affected the increasing of the little sperm pipes and the proliferation of male gonocytes (HAN ZENGKANG, 1980). Therefore, the functions of the altered testicle tissue got recovered (the size of the testes became larger), promoting the production and maturation of the spermatozoa as well as the secretions of the sexual annex glands when LRH-A_2 was injected.

Being a natural mineral mixture of lanthanum metals, rare-earth is a kind of nitrated matter. It is also named "the industrial gourmet powder" since it is now being widely used in industry, national defence, medicine and agriculture, etc. It could be used both in agriculture and animal husbandry to promote the output and quality of the plants and increase the rates of egg-laying as well as the weight of the chicken (TANTAI BINGYAN, 1989). Some experiments made by the author and other scholars showed that rare-earth could increase milk secretion and improve the output and quality of the female rabbits, but the mechanism hasn't been elucidated yet (WANG MAO, 1989; GU ZILIN, 1991). It was thought that rare-earth had something to do with enzymes in the body and it was the components or activators of some enzymes, catalysing reactions in the animal bodies

(ZHENG JUN, 1990). It was shown from this experiment that the using of LRH-A_2 alone or the combination of LRH-A_2 with rare-earth could both induce a better testicular function of male rabbit but a much better effect could be got when the two were applied together. It was obvious that the two could have a kind of co-ordinated function.

As for the problem of no significant improvement of the testicular function for the rabbits in the control group at the end of the experiment, we thought it was perhaps influenced by the factors such as nutrition, season and clipping. The experimental diet provided by the farm was imbalanced and of low nutritional value, and the grass quality was prominently reduced though one rabbit was given 400g a day. Moreover, that the rabbits were clipped at that time caused the nutrition was mainly used to their wool's growth and this used for the testes was relatively reduced. In the last place, it was in late autumn, the sunshine period was shorter; the average temperature in the experiment was 22.4°C while the highest temperature was 32.6°C. All this was bad for the recovering of the rabbits' testicular function. The same effect was found when the author measured the rabbits testicles in other farms, in the same period of time.

It was demonstrated both abroad and in China that high temperature would bring bad effects to the testicular function of the male rabbits. Both the alteration of the epithelium of testicles and the temporary loss of the function of spermatozoa making were caused by high temperature. It was believed that the appetite would decrease, the weight would become less and the normal metabolism would be badly influenced by high temperature of summer. This would damage the sperm making function of the male rabbits (DU YUCHUAN, 1987). Through the experimental results and some production observations, the authors thought that the following three points caused bad effects to the male rabbit's testicles suffering from heat stress :

- The synthesis and production of the releasing hormone (GnRH) of the ovestrin in the inferior colliculus in the brain was restricted under high temperature, thus, controlled the LH and FSH of the pituitary as well as the synthesis and release of the testosterone.
- The nutrients input in the testes became lower because the male rabbits ate less food under high temperature.
- The body position and the increase of the blood's rate of flow in the outer parts of the body would help to release heat; in the other hand, this decreased the supply of blood to the internal organs of the body, decreasing the motility of the cells as well as the production and release of the heat. So, the tissue of the testes would gradually decline since the blood supply to the testes was not enough for a long time, and the testes were always in a state of hunger.

The experiment showed that inoculating LRH-A_2 and adding rare-earth in the feed of the rabbits suffering of heat stress could get following effects : their testicular function could be recovered ; their testicular tissue proliferated; their ejaculate, sperm density and motility increased; the conditions of no or dead spermatozoa production disappeared, their sexual desire and the pregnancy rates were both increased but no side effect was found for the treated rabbits. The function of LRH-A_2 was its reparation to the shortage of GnRH's secretion but the mechanism of rare-earth has not been elucidated yet. The authors believed that rare-earth had something to do with some enzymes in the body and accelerated the relative biochemical reactions within the body being the important components of the enzyme or being a kind of activator.

The experiment on the effects of LRH-A_2 and rare-earth to the testicular functions of the rabbits suffering of heat stress was made in bad conditions of nutrition. Better results could be got if the full maintenance feed was provided. Owing to the limitation of production conditions, only some external indexes of the testes and routine indexes of the seminal fluid were measured but the variations of the testicular tissues and those of hormones and enzymes in the blood have not been tested.

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LRH-A_2、稀土对热应激安哥拉公兔睾丸机能的影响

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摘　要

选择热应激唐行系安哥拉公兔32只，随机分成4组，每组8只。试验一组肌注LRH－A_2，每3天一次，每次5mg，5次后改为5天一次，共注8次。试验二组饲料中加200ppm的稀土。试验三组肌注LRH-A_2（同一组）和饲喂稀土日粮（同试验二组）。对照组不予处理。历时一个月。各组兔日粮相同，每只日喂150克，同时补喂青草400克。分别于试验开始（1991年9月5日）和结束（1991年10月5日）测定睾丸体积（睾丸长×宽×厚），采集精液，测定射精量、精子密度及活率，并每组随机配种（人工授精）30只母兔，观察配种受胎率。结果表明：三个试验组睾丸分别比试验前增大9.94%、5.37%和16.71%（$P>0.05$），而对照组缩小了26.56%（$P<0.05$）；四个组公兔射精量分别增加了0.28ml、0.31ml、0.24ml和0.13ml；精子密度增加了0.1、0.07、0.65和0.1亿/ml；精子活率分别提高0.23、0.24、0.42和0.09。母兔受胎率分别为19、20、23和11只，配种受胎率分别为63.33、66.67、76.67和36.67%。均以试验三组效果最佳。试验初步表明，LRH-A_2和稀土元素对热应激公兔睾丸机能恢复有较好效果，二者配合使用有协同作用。

关键词：安哥拉公兔；LRH-A_2；稀土；热应激；睾丸机能