Comparative Analysis of Semen between Debao Pony and American Pearl Pony

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Abstract ［**Objectives**］The demand for ponies has been increasing in recent years，but the stock of Debao ponies is far from enough to supply market demand. In this context，this paper aims to achieve the purpose of conservation，purification and rejuvenation. ［**Methods**］Under the conditions of same age，semen collection frequency，season，feeding environment，feed nutrition level，*etc*.，the semen of Debao pony and American pearl pony were collected，respectively，and the quality of the semen collected was analyzed. ［**Results**］There was no significant difference in semen volume between the two breeds of ponies；the sperm vitality of the firstly-collected semen of ponies of which the semen has not been collected for a long time was low. The semen quality of American pearl pony was slightly better than that of Debao pony. ［**Conclusions**］When the semen quality of American pearl pony is stable，it can be used for cross-breeding with the Debao pony mare，highlighting the hybrid vigor of the two breeds，thereby achieving the purpose of reducing the size of ponies.

Key words Debao pony，American pearl pony，Semen，Comparison

1 Introduction

In recent years，with the rise of horse industry of entertainment and sport types，the proportion of ponies，as the emerging tertiary horse industry，in recreational riding competitions is increasing rapidly year by year. Debao Pony is one of the two largest pony sources in the world，mainly produced in Debao，Guangxi. Its average body height is only about 100 cm，and the shortest is only 85 cm. According to research，it is a descendant of the Guoxia Pony in the Western Han Dynasty. One of the most distinctive characteristics of Debao pony is pure color，mainly including brownish black，brownish red，light brown，grayish yellow and grayish white. Debao pony is characterized with drawfness，beautiful body，gentle temperament，dexterity，piggyback tolerance，and strongness. For a long time，Debao ponies are scattered in farmers' homes and are bred in the form of natural grazing，lacking unified management and systematic scientific selection work.

At present，although the Debao County Animal Husbandry Bureau has carried out a little work on centralized breeding and registration of Debao pony，the data are incomplete. Funds and certain measures are not in place. Farmers can only receive little economic benefit，so their enthusiasm for feeding is not high. The stock of Debao pony has not been increased for a long time，and instead，it shows a sharp decline trend. Debao pony currently has a stock less than 2 000 heads，and the protection of germplasm resources of new pony species is on the verge of danger[1]. In this

context，it is necessary to adopt active protective measures and industrialization strategies，carry out basic research on the genetic law，physiology and molecule of Debao pony in Guangxi，increase investment in the breeding and selection of Debao pony，adopt systematic engineering method to carry out pure breeding between excellent individuals and introduce American pearl pony for hybrid breeding to achieve the purpose of conservation，purification and rejuvenation，and establish genetic resources conservation bases and protected areas for Debao pony as soon as possible[2]. In order to provide a research basis for carrying out cross-breeding experiments on Debao pony and American pearl pony，in this paper，the semen of these two breeds was analyzed and compared comprehensively.

2 Materials and methods

2.1 Test animals Two Debao pony stallions were selected. One was white，and named as Xiaobai. It was 10 years old，90 cm tall，86 kg in weight，healthy and moderate in fatness. The other Debao pony stallion was named as Heihei. It was 11 years old，96 cm tall，90 kg weight，healthy and moderate in fatness. One American pearl pony stallion was selected. It was named as Dudu，11 years old，76 cm tall，73 kg in weight，healthy and good in body condition.

The experiment was conducted in the Debao pony base of Animal Husbandry Research Institute of Guangxi Zhuang Autonomous Region. All the ponies test were kept in separate peens with the same feed. According to body weight and body condition，the amount of feed given to Debao ponies was higher，including 10 kg of forage and 2.0 kg of concentrated feed. The American pearl pony Dudu has good body condition. It was short，and its daily feed intake was composed of 8 kg of forage and 1.5 kg of concentrated feed. During the experiment，after each semen collection，one raw egg was supplemented to the diet of every stallion per day，and the interval between every two adjacent

times of semen collection was 7 d.

2.2 Artificial semen collection Good preparations were made before semen collection. The artificial vagina used was a small one for bull semen collection. As the penises of Debao ponies were thicken and longer than that of American pearl pony, the artificial vagina was fully inflated when used for American pearl pony, and could not be fully inflated when used for Debao ponies. The normal body temperature of ponies is $37.5 - 38.5$ ℃. According to the principle that the storage temperature of semen should be close to body temperature, the temperature of the hot water injected into the artificial vagina was maintained at $40 - 42$ ℃. Before use, a proper amount of paraffin oil was applied to the opening of the artificial vagina to lubricate. After the preparation was completed, a mare in estrus was fixed to the hold flame, and one person helped to fix the mare. As each stallion crawled on the mare, its penis was exposed and ready to enter the mare's vagina. When approaching, the semen collector quickly used the artificial vagina to cover the stallion's penis. After holding tightly, the other hand of the semen collector held the back of the stallion to help fix and feel the stallion's movements. After feeling that the stallion's penis softened and retracted, the artificial vagina was pulled away from the penis. The semen in the collection tube was taken out, and its volume was measured and recorded. The contaminated semen on the surface was drained off, and the remaining was poured into a sterile test tube, which was wrapped with a clean towel to keep warm ($39 - 40$ ℃) and in dark and then sent to the laboratory quickly for inspection.

2.3 Detection methods and indicators The semen samples were sent to the laboratory and preserved in a constant-temperature water tank. After the sperms recovered vitality, the semen samples were inspected using high-power electron microscope and semen density measuring instrument. The indicators inspected include semen volume, semen appearance color, sperm motility, sperm deformity index and sperm density.

3 Results and analysis

The semen of the two breeds of ponies had a special fishy smell. The color of the semen was milky white or milky grayish white. Under a high-power electron microscope, it could be observed that the linearly-moving sperms of American pearl pony were more and faster than those of Debao pony; the average sperm deformity index of Debao pony and American pearl pony was $64.9\% - 69.8\%$ and 36.3%, respectively; and the sperm tail of American pearl pony was longer than that of Debao pony (Table 1). The inspection results of the first semen collection of Debao pony and American pearl pony were both not satisfactory, and the quality of the semen was very poor. A large number of dead sperms were observed in the semen of Xiaobai, and the semen of Heihei was basically normal. After a week of rest and raw egg feeding, the quality of the semen was improved significantly. The results of the second test were better than those of the first time. The semen volume, sperm mobility and sperm density of American pearl pony were all higher than those of Debao pony. In the follow-up inspections, the results of various indicators were relatively stable (Table 2).

Table 1   Motion analysis of sperms of the tested ponies

Test pony	Normal movement		Abnormal movement	
	Sperm quantity	Proportion %	Sperm quantity	Proportion %
Debao pony (Xiaobai)	76	30.2	177	69.8
Debao pony (Heihei)	75	35.1	140	64.9
American pearl pony (Dudu)	150	63.7	86	36.3

Table 2   Semen quality inspection results of the tested ponies

Test pony	Inspection No.	Semen volume // mL	pH	Semen color	Fluidifying time // min	Sperm motility	Sperm density // 10^8/mL	Deformity index // %
Debao pony (Xiaobai)	1	25	7.2	Milky white	25	0.10	1.36	85.0
	2	28	7.1	Milky white	22	0.25	1.43	70.0
	3	28	7.1	Milky white	25	0.24	1.45	67.0
	4	32	7.2	Milky white	24	0.25	1.48	65.0
	5	28	7.2	Milky white	25	0.24	1.42	62.0
Debao pony (Heihei)	1	25	7.1	Milky grayish white	24	0.21	1.45	67.1
	2	25	7.3	Milky grayish white	25	0.23	1.51	65.3
	3	26	7.3	Milky grayish white	25	0.22	1.53	64.5
	4	25	7.2	Milky grayish white	25	0.25	1.53	60.6
	5	27	7.3	Milky grayish white	25	0.24	1.58	61.2
American pearl pony (Dudu)	1	28	7.5	Milky white	23	0.20	1.60	39.5
	2	32	7.4	Milky white	23	0.35	1.64	36.7
	3	29	7.5	Milky white	23	0.29	1.61	35.2
	4	31	7.4	Milky white	22	0.35	1.64	34.7
	5	32	7.5	Milky white	23	0.32	1.62	35.8

4 Conclusions and discussion

(ⅰ) The results of this study show that the difference in semen volume of the two breeds of ponies was not significant. The sperm

pects and perspectives.

5.3 Strengthening multi-channel real-time feedback mechanism to realize three-dimensional interaction and continuous communication between teachers and students   Teachers can actively transmit teaching resources to students through the intelligent platform, and they can immediately review and feed back the students' homework and learn about the students' situation in time. Students can also receive course information anytime and anywhere for autonomous learning. In addition to classroom communication, we can also use the cloud platform to communicate outside the classroom anytime and anywhere, to achieve three-dimensional interaction and all-round continuous communication between teachers and students, between students beyond time and space[8].

5.4 Changing the learning style of learners and paying attention to personalized creative learning   The mixed teaching model emphasizes that teachers are only the guides of learning and students are the center. It encourages students to cooperate in team learning, encourages students to carry out independent exploratory learning and creative learning with the help of intelligent platform, cultivate students' ability to analyze and evaluate problems, and cultivate students' innovative thinking and creativity, promote students' own transcendence and innovation.

5.5 Forming diversified teaching evaluation and evaluating the learning effect of learners from multiple levels and multiple angles   It is necessary to change the traditional one-sided teaching evaluation system, focus on the comprehensive evaluation of students' learning effect from the aspects of students' knowledge mastery ability, autonomous learning ability, cooperation ability, problem-solving ability and so on, and pay attention to the diversification of evaluation methods and evaluation content.

6 Conclusion

Under the tide of "Internet + education", the intelligent class based on the new generation of information technology is the product of the deep integration of new technology and education. The construction of mixed teaching model in colleges and universities in the context of "Internet +" effectively integrates the advantages of online teaching and traditional teaching, and realizes the deep integration of Internet and teaching model. This is not only a new idea of education and teaching reform in colleges and universities, but also an effective attempt to change students' learning methods and teachers' teaching methods by means of intelligent technology.

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motility and sperm deformity index of the first semen collection of ponies of which semen had not been collected for a long time were relatively low. Under the condition of balanced nutrition level, it is recommended to conduct semen collection for 1−2 times. After all the indicators are qualified for microscopic examination, semen can be divided into tubes and frozen to be used for artificial insemination or crossbreeding. (ii) When the semen quality of American pearl pony is stable, it should be bred with Debao pony mare to highlight the hybrid vigor of the two breeds and achieve the effect of reducing the body size of the ponies. (iii) The semen quality of American pearl pony is slightly higher than that of Debao pony. But compared with the quality of semen collected before, the quality of semen inspected this time is not high. There are many reasons. First, the semen collection and inspection was performed in August. In the hot season, due to the effect of heat stress, the quality of pony semen was not high. Second, these three ponies had never performed artificial semen collection before. For the first semen collection of animals, the quality of semen is mostly low, manifested by low vitality, high death rate, high deformity rate and low sperm density. Third, the artificial vagina used in this study was specific to bull semen collection. For semen collection of stallion, it is best to use horse-specific sperm collection equipment and models, in order to improve the comfort and ejaculation of the stallion. Fourth, the thinner used in this study was specific to bull semen, and whether its osmotic pressure had an impact on the ponies' semen was not clear. Under a high-power microscope, it was observed that there were certain differences in the sperms of Debao pony and American pearl pony, i. e., the morphology and movement speed of sperms of Debao pony and American pearl pony were slightly different. Whether these differences were caused by the difference between the two breeds requires further research.

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