Analysis and Utilization of Straw-preserved Frozen Semen from Native and Imported Pig Breeds in Guizhou Province

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Abstract [**Objectives**] Straw-preserved frozen semen from native and imported pig breeds and its conception performance were studied to provide a scientific basis for further optimizing pig breeding strategy. [**Methods**] This study was conducted to systematically evaluate straw-preserved frozen semen produced by the Guizhou Testing Center for Livestock and Poultry Germplasm from 2018 to 2023. We analyzed 206 samples from native Guizhou pig breeds, including Kele, Zongdi Hua, and Nuogu pigs, as well as 363 samples from imported and bred pig breeds, such as Duroc, Large Yorkshire, Landrace, Topigs, and French Yorkshire, among others. Additionally, we assessed the conception performance of more than 300 heads across 10 batches, comprising both native and imported and bred pig breeds, following deep insemination. [**Results**] Significant differences were identified in sperm motility and abnormal sperm percentage (ABSP) among native breeds ($P<0.05$), as well as in number of progressively motile sperm (NPMS) and ABSP among imported and bred breeds ($P<0.05$). The average semen dose for both native and imported/bred pigs was 0.47 ml, with a standard deviation of 0.01 ml. Sperm motility ranged from 41.5 % to 58 % in native breeds and 50.4 % to 61.7 % in imported and bred breeds. The NPMS ranged from 144 to 177 million in native breeds and 149 to 177 million in imported and bred breeds. ABSPs remained within acceptable limits, ranging from 6.6 % to 16.8 % for native breeds and 6.1 % to 18.1 % for imported and bred breeds. The imported and bred pigs outperformed the native breeds in terms of sperm motility (50.42 % to 69.58 % vs. 41.63 % to 48.37 %), farrowing rate (71.13 % to 86.70 % vs. 57.15 % to 74.35 %), and litter size (7.74 to 10.30 vs. 6.39 to 7.45). [**Conclusions**] These findings suggest that long-term selective breeding and domestication of native Guizhou pigs are necessary to enhance their reproductive performance.

Key words Pig frozen semen; Sperm motility; Number of progressively motile sperm(NPMS); Abnormal sperm percentage(ABSP); Conception rate

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Pig breeding industry is the core and source of high-quality development of pig industry. The frozen semen of excellent breeding boars can effectively solve the problems of large number of breeding boars, high feeding cost and high epidemic prevention pressure[1-2]. Compared with normal-temperature semen, frozen semen has obvious advantages in storage, transportation, use areas, disease resistance, breeding cost saving and breeding intensity enhancement. However, due to the special structure of pig sperm, the mechanism of pig sperm freezing is not clear enough compared with animals such as cattle and sheep, and the efficiency is low[3-4], and it is difficult to popularize and apply because of the lack of freezing reagents, thawing reagents and freezing equipment suitable for production. In recent years, studies have shown that the freezing tolerance of pig semen is related to pig breeds. The toxicity and osmotic pressure changes of cryoprotectants, freezing and cold shock during freezing and thawing processes[6], and excessive ROS production during freezing and thawing of boar sperm can lead to impaired sperm motility, plasma membrane, mitochondrial activity and sperm chromatin structure, and cell apoptosis[7]. Frozen diluents can improve sperm motility after thawing of frozen pig semen and sow reproductive performance[8]. Large-scale breeding enterprises, pig breeding bases and related scientific research institutions have gradually established the production and use system of frozen semen and mastered the techniques of semen collection, treatment, freezing and preservation, and can provide high-quality frozen semen.

According to the latest research results and production practice, in this study, the frozen semen of native Guizhou pig breeds and imported and bred pig breeds and its conception performance were investigated, aiming to provide a scientific basis for further optimizing pig breeding strategy.

Materials and Methods

Experimentlal materials

Frozen semen from breeding pigs: Frozen semen was produced from native Guizhou pig breeds, including Kele, Zongdi Hua, and Nuogu pigs, as well as imported and bred pig breeds, such as Duroc, Large Yorkshire, Landrace, Topigs, and French Yorkshire among others from Guizhou breeding pig demonstration farm. Conception performance: An insemination experiment was carried out using straw-preserved frozen semen of breeding pigs in Guizhou Wucui Mingxiang Agricultural Development Co., Ltd., and the results of insemination were recorded.

Experimental methods

Preparation of frozen semen Production process of frozen

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semen: First, fresh semen was diluted with liquid I (at 17 ℃) in a ratio of 1:1. After standing at room temperature (about 25 ℃) for 1 h, it was transferred to a balance box at 17 ℃ for at least 2 h (<24 h). Next, it was centrifuged at 900 times the gravitational force (900 g) at 4 ℃ for 8 – 9 min. The supernatant was discarded, and the semen was re-suspended in liquid I to achieve a sperm density of 2 billion/ml. The sperm volume was estimated using the weighing method. After slowly cooling down to 4 ℃, equal volume of liquid II was added at the same temperature to make the sperm density 1 billion/ml, and the obtained liquid was mixed well. Subsequently, automatic filling was carried out at 4 ℃ using a 0.5 ml straw. Gradient freezing was carried out by using a programmed freezer. The temperature was reduced from 4 to 1 ℃ within 2 min, then to −140 ℃ within 4.7 min, and kept at −140 ℃ for 5 min. The prepared frozen semen was then stored in liquid nitrogen.

Results and Analysis

Detection results of pig frozen semen

We systematically determined and evaluated frozen semen produced by the Guizhou Testing Center for Livestock and Poultry Germplasm from 2018 to 2023, including 206 samples from native Guizhou pig breeds, as well as 363 samples from imported and bred pig breeds. The evaluation focused on following parameters: frozen semen dose, sperm mobility, number of progressively motile sperm (NPMS), and abnormal sperm percentage (ABSP). The results are as follows:

From the data analysis in Table 1, it can be seen that, the first term was the dose of pig frozen semen, and there was no significant difference in the dose of pig frozen semen samples ($P>0.05$). At present, the average filling volume of straw volume in pig frozen semen production process can reach 0.47 ml, and the standard deviation is about 0.01 ml. Under the standardized production conditions, the filling process of frozen semen is basically in a controlled and stable state, and there is no significant difference in sample dose. The second was sperm motility, which is the key test item of pig frozen semen quality. There were significant differences in sperm motility among native breeds ($F=7.145$, $P=0.002$), but no significant difference was observed among imported breeds ($P=0.302$), indicating that the sperm motility of imported breeds was lower and more uniform than that of native breeds. The third was NPMS. There were significant differences in NPMS among introduced breeds ($F=2.271$, $P=0.038$), but there was no significant difference among native breeds ($P=0.067$). The fourth was ABSP. No matter native breeds or imported breeds, their ABSPs showed significant differences, but the differences for native breeds were even greater (native breeds $F=85.888$, $P\leqslant 0.001$; imported breeds $F=4.563$, $P\leqslant 0.001$). Generally speaking, there were significant differences in sperm motility and ABSP among native breeds, while imported breeds exhibited significant differences in NPMS and ABSP.

Table 1 One-way ANOVA results of frozen semen dose, sperm viability, NPMS and ABSP

Item	Type	Sum of squares	*df*	Mean square	*F*	Significance
Dose//ml	Among native breeds	0.002	8	0	1.748	0.088
	Among imported breeds	0.001	6	0	1.048	0.395
	Within group	0.041	561	0		
	Total	0.043	569			
Sperm mobility//%	Among native breeds	2 564.191	2	1 282.096	7.145	0.002
	Among imported breeds	2 354.794	6	392.466	1.212	0.302
	Within group	72 053.543	561	302.746		
	Total	77 211	569			
NPMS//1.0×10^8	Among native breeds	1.129	2	0.565	2.843	0.067
	Among imported breeds	73.044	6	12.174	2.271	0.038
	Within group	80.45	561	0.338		
	Total	81.898	569			
ABSP	Among native breeds	1 073.221	2	536.611	85.888	≤0.001
	Among imported breeds	512.254	6	85.376	4.563	≤0.001
	Within group	5 063.123	561	21.274		
	Total	5 910.838	569			

Sperm motility analysis of pig frozen semen

In this study, the key quality index "sperm motility" of pig frozen semen was statistically analyzed. Sperm motility is defined as the recovery rate of sperm after thawing (%), that is, the percentage of progressively motile sperm to the total sperm count[9]. Specific indicators are shown in Table 2.

According to the analysis results in Table 2, the sperm motility of native Guizhou breeds ranged from 41.5% to 58%, with an average of 50.4% and an average deviation of 14.8%. Among them, Kele pigs showed the highest sperm motility at 58.0%, and exhibited smallest individual differences and a standard deviation of 4.8%. The sperm motility of Nuogu pigs was lowest at 41.5%, and showed largest individual differences and a standard deviation of 21.6%. The value of Zongdi Hua pigs was at the middle level. In contrast, the sperm motility of imported and bred breeds ranged from 50.4% to 61.7%, with an overall average of 53.9% and an

average deviation of 18.1%. Topigs performed the best, with an average sperm motility of 61.7%, and the individual differences were small, and the standard deviation was 9.0%. Generally speaking, the imported and bred breeds showed higher sperm motility overall, so they have more advantages in the application of frozen semen technology, which may be related to genetic background and breeding technology. Among them, native Guizhou breed Kele pigs and imported Topigs showed relatively highest sperm motility and smaller individual differences, indicating good breeding potential.

Table 2 Detection results of sperm motility of straw-preserved pig frozen semen %

No.	Breed	Sample number N	Mean	Standard deviation	Standard error	95% confidence interval of the mean		Minimum	Maximum
						Lower limit	Upper limit		
Native Guizhou breeds									
1	Kele pigs	70	58.0	4.8	1.39	54.95	61.05	47.4	64.3
2	Zongdi Hua pigs	70	50.8	8.9	2.57	45.15	56.45	36.0	62.2
3	Nuogu pigs	66	41.5	21.6	6.24	27.78	55.22	30.1	65.8
Total		206	50.4	14.8	4.27	41.00	59.80	30.10	65.8
Imported and bred pig breeds									
1	Duroc	60	50.4	20.6	5.95	37.31	63.49	47.4	81.8
2	Large Yorkshire	60	52.6	21.8	6.29	38.75	66.45	49.9	90.2
3	Landrace	60	55.6	13.6	3.93	46.96	64.24	50.1	68.2
4	Topigs	60	61.7	9.0	2.60	55.98	67.42	51.9	85.2
5	French Yorkshire	60	57.3	3.6	1.04	55.01	59.59	52.4	55.3
6	Others	63	53.9	2.1	0.61	52.57	55.23	50.6	61.7
Total		363	53.9	18.1	5.23	42.40	65.40	47.4	90.2

Analysis on NPMS in pig frozen semen "NPMS" directly affects the conception rate. It refers to the number of sperm that can move forward along a straight line or a relatively straight track in a semen sample, and is an important index for evaluating semen quality. Generally speaking, the content of imported breeds in breeding is 2×10^8 or more.

According to the analysis results in Table 3, among the 206 pigs from native Guizhou breeds, the NPMS per dose ranged from 144 to 177 million, with an average of 165 million. Among them, the average sperm count of Kele pigs was 177 million, but the standard deviation was 56 million, showing great individual differences. The value of Zongdi Hua pigs was 171 million, which was in the middle, and the individual differences were small. By comparison, the average number of Nuogu pigs was the lowest at 144 million. Among the 363 pigs from imported and bred pig breeds, the NPMS per dose ranged from 149 to 177 million, with an average of 170 million. Among them, the NPMS in both Duroc and Large Yorkshire was 170 million, but the individual differences were relatively large. Landrace performed the best, with an average of 177 million, but the individual differences were slightly higher. However, French Yorkshire showed the lowest value and small individual differences. Generally speaking, although there were small differences in the average value and 95% confidence interval between native Guizhou breeds and imported and bred pig breeds, but there were individuals with extremely low NPMS in Kele pigs and Nuogu pigs.

Table 3 The results of NPMS in straw-preserved frozen semen of imported and bred pig breeds 1.0×10^8

No.	Breed	Sample number N	Mean	Standard deviation	Standard error	95% confidence interval of the mean		Minimum	Maximum
						Lower limit	Upper limit		
Native Guizhou breeds									
1	Kele pigs	70	1.77	0.56	0.16	1.41	2.13	0.51	2.76
2	Zongdi Hua pigs	70	1.71	0.24	0.07	1.56	1.86	1.30	2.20
3	Nuogu pigs	66	1.44	0.48	0.14	1.14	1.74	0.53	2.30
Total		206	1.65	0.46	0.13	1.36	1.94	0.51	2.76
Imported and bred pig breeds									
1	Duroc	60	1.70	0.74	0.21	1.23	2.17	1.00	3.60
2	Large Yorkshire	60	1.70	0.59	0.17	1.33	2.07	1.00	2.82
3	Landrace	60	1.77	0.54	0.16	1.43	2.11	1.12	2.85
4	Topigs	60	1.63	0.33	0.10	1.42	1.84	1.21	2.42
5	French Yorkshire	60	1.49	0.27	0.08	1.32	1.66	1.00	1.85
6	Others	63	1.53	0.01	0.00	1.52	1.54	1.52	1.54
Total		363	1.70	0.59	0.17	1.33	2.07	1.00	3.60

Analysis on ABSP of pig frozen semen

Reasonable control of ABSP can help to improve the quality of pig frozen semen, reduce waste rate, and avoid waste of genetic resources. Generally speaking, the ABSP of straw-preserved pig frozen semen is no more than 20%, which is related to semen itself.

According to the analysis results in Table 4, the ABSPs of native Guizhou breeds ranged from 6.6 % to 16.8 %, with an average of 12.9%. In specifiic, the average value of Nuogu pigs was 6.6%, which showed the best sperm morphological quality, but there were great differences among individuals. The average values of Kele pigs and Zongdi Hua pigs were, respectively, 16.8% and 14.6%, which were relatively higher, and the individual differences were greater. The ABSPs of imported and bred pig breeds ranged from 6.1% to 18.1%, with an average of 9.0%. The average values of Duroc and Large Yorkshire were 9.9% and 9.0%, respectively, showing good stability. The average value of Topigs was 8.0, and the individual differences were the smallest. The average value of other breeds was 18.1%, and they showed great individual differences. Generally speaking, there were significant differences between native Guizhou breeds and imported and bred pig breeds in the morphology of straw-preserved frozen semen, but the ABSPs all remained within acceptable limits.

Table 4 The results of ABSP in straw-preserved frozen semen of imported and bred pig breeds %

No.	Breed	Sample number *N*	Mean	Standard deviation	Standard error	95% confidence interval of the mean		Minimum	Maximum
						Lower limit	Upper limit		
Native Guizhou breeds									
1	Kele pigs	70	16.8	0.8	0.33	15.96	17.64	15.2	17.6
2	Zongdi Hua pigs	70	14.6	1.8	0.73	12.71	16.49	12.3	18.1
3	Nuogu pigs	66	6.6	4.0	1.63	2.40	10.80	2.2	18.3
Total		206	12.9	5.0	2.04	7.65	18.15	2.2	18.3
Imported and bred pig breeds									
1	Duroc	60	9.9	4.3	1.76	5.39	14.41	2.9	18.3
2	Large Yorkshire	60	9.0	4.4	1.80	4.38	13.62	2.2	18.7
3	Landrace	60	8.4	4.7	1.92	3.47	13.33	2.4	18.6
4	Topigs	60	6.1	3.3	1.35	2.64	9.56	2.2	15.4
5	French Yorkshire	60	12.8	3.6	1.47	9.02	16.58	7.3	18.7
6	Others	63	18.1	0.9	0.37	17.16	19.04	7.5	17.4
Total		363	9.0	4.5	1.84	4.28	13.72	2.2	18.7

Analysis on conception rate of pig frozen semen

Through experiments, it has been found that the farrowing rate of pig frozen semen by conventional insemination mode is extremely significantly lower than that by deep insemination mode. Therefore, the deep insemination mode was adopted. We assessed the conception performance of more than 300 heads across 10 batches, comprising both native pig breeds (Nuogu pigs and Kele pigs) and imported and bred pig breeds (Large Yorkshire, Duroc and Landrace), following deep insemination. The details are shown in Table 5.

Table 5 Single sample t-test statistics of deep insemination using frozen semen from native Guizhou pig breeds and imported pig breeds

Type	Index	*t*	*df*	Sig. (two-sided)	Mean	95% confidence interval of the mean	
						Lower limit	Upper limit
Native Guizhou breeds	Sperm motility//%	30.187	9	0	45.00	41.63	48.37
	Farrowing rate//%	17.296	9	0	65.75	57.15	74.35
	Litter size	29.633	9	0	6.92	6.39	7.45
Imported and bred pig breeds	Sperm motility//%	13.787	11	0	60.00	50.42	69.58
	Farrowing rate//%	22.315	11	0	78.92	71.13	86.70
	Litter size	15.499	11	0	9.02	7.74	10.30

In the process of deep insemination, the native Guizhou breeds showed sperm motility ranging from 41.63% to 48.37%, farrowing rate ranging from 57.15% to 74.35%, and litter size ranging from 6.39 to 7.45. The imported breeds exhibited perm motility ranging from 50.42% to 69.58%, farrowing rate ranging from 71.13% to 86.77%, and litter size ranging from 7.74 to 10.30. It could be seen that there were significant differences between native Guizhou breeds and imported breeds in terms of frozen sperm motility, farrowing rate and litter size, and imported breeds had more advantages. Among the various indicators, the frozen sperm motility of imported and bred breeds had a significant impact on litter size.

Conclusions and Discussion

To sum up, the frozen semen of native Guizhou breeds and imported and bred breeds can reach a certain quality and application level. Native Guizhou breeds: The sperm motility of Kele pigs was highest (58.0%), and the individual differences were smallest. Nuogu pigs showed the lowest sperm motility (41.5%) and largest individual differences, and the value of Zongdi Hua pigs was in the middle. Kele pigs showed the highest NPMS (177 million), but the individual differences were great. Zongdi Hua pigs

exhibited moderate NPMS (1.71 million), and the individual differences were small. The ABSP of Nuogu pigs was lowest (6.6%), with great individual differences. The ABSPs of Kele pigs and Zongdi Hua pigs were higher, at 16.8% and 14.6% respectively, and the differences among individuals were small. Imported and bred breeds: Topigs showed highest sperm motility (61.7%), with smallest individual differences. Landrace pigs had the highest NPMS (177 million), but there were great individual differences. French Yorkshire performed the worst, and the individual differences were small. The ABSPs of Duroc and Large Yorkshire were 9.9% and 9.0%, respectively, showing stable performance. Topigs exhibited lowest ABSP (8.0%) and smallest individual differences. The ABSPs of other breeds were higher (18.1%), and they showed great individual differences. There were significant differences in sperm motility and ABSP among native breeds ($P < 0.05$), while imported breeds showed significant differences in NPMS and ABSP ($P < 0.05$). Imported and bred breeds with strict breeding and excellent genetic background showed significant advantages in sperm motility of frozen semen and have higher breeding efficiency. In contrast, native Guizhou breeds had strong adaptability in specific environment, but there were great differences among individuals. In order to improve semen quality and overall reproductive efficiency, breeding management and technical optimization should be strengthened, and long-term qualitative selection and domestication should be carried out. Molecular genetic methods can also be considered to improve pig breeds that are not tolerant to freezing[10]. In addition, because the age and daily management of boars also affect the quality of semen to a certain extent, the scale of experiments should be further expanded to reduce the influence caused by other factors, and frozen semen may have an impact on the genetic performance of pigs[12], so further relevant research can be carried out.

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(Continued from page 16)

decreased, while in the habitat outside the nature reserves such as Baoxing County, Xiaojin County and Tianquan County in the middle and south sections of the mountains, the activities of giant pandas were frequent. Therefore, it is necessary to expand the protected areas in these areas or build new protected areas of giant pandas, so as to prevent the impact of human activities on the giant panda population. The comparative analysis on the avoidance effect of giant pandas on partial disturbances showed that there were significant differences in the avoidance effect of partial disturbances between the two surveys, including logging, grazing, hunting, highways and other types of disturbances.

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