Characteristics of the Ejaculates from Meishan Boars at Various Ages

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Abstract The age at which ejaculatory ability is achieved and age-related changes in the characteristics of ejaculates were examined in Meishan boars. All of the 12 boars examined showed detachment of the penis from the prepuce by 64 days of age. The first ejaculate could be collected at 73 to 78 days of age. The volume of the liquid portion, the weight of the gelatinous material, the total number of spermatozoa per ejaculate and the sperm concentration increased significantly until 11, 10, 9 and 5 months of age, respectively. Percentages of progressively motile spermatozoa and morphologically abnormal spermatozoa in the ejaculates altered greatly until 3 months of age, and thereafter stayed at a good level. Percentages of live spermatozoa with normal acrosomes after storage at 4°C for 5 and 10 days were higher in ejaculates collected from boars older than 9 months, and percentages of progressively motile spermatozoa and live spermatozoa with normal acrosomes after freeze-thawing in boars older than 8 months. These results suggest that Meishan boars acquire ejaculatory ability at approximately 2 months of age, but should not be used in service until later ages. It is also suggested and that ejaculates from boars younger than 9 months are less suitable for storage at 4°C -196°C.

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Meishan pigs are noted for their early puberty^{1,18,24)}. We⁶⁻⁸⁾ have reported that the rapid development of the reproductive organs of Meishan boars occurred after 45 days of age. Spermatozoa first appear in the rete testicular and epididymal fluids at approximately 75 days of age^{6,7,9)}. The ability of the testis to produce morphologically normal spermatozoa and that of the epididymis to mature spermatozoa are established by 120 days of age^{6,9)}. The secretory activity of the seminal vesicle, prostate and bulbourethral gland becomes active after about 2, 2 and 1 months of age, respectively⁸⁾. To our knowledge, however, no detailed study

on ejaculatory ability and age-related changes in the characteristics of ejaculates in Meishan boars has been reported. The objectives of the present study were to determine the age at which boars acquire ejaculatory ability and to characterize the ejaculates collected from the same boars at various ages.

Materials and Methods

Animals : A total of 15 Meishan boars were used. They were housed under the same conditions as previously described¹⁸⁾, except that the formula feed for the breeder (Nihon Nosan Kogyo Co., Yokohama, Japan) was

given after 81 days of age.

Examination of the detachment of the penis from the prepuce: Twelve boars were given a sedative containing 20–40 mg of 4'-fluoro-4-[4-(2-pyridyl)-1-piperazinyl] butyrophenone (Stresnil, Sankyo Co., Tokyo, Japan) per head by an intramuscular injection every 5 days. The boar whose penis appeared out of the preputial orifice after injection was counted as an animal with the penis detached from the prepuce.

Collection of ejaculates and examination of their characteristics: Two groups of 3 boars each were used. In Group A, boars were trained to mount a small-sized dummy at less than 70 days of age. In Group B, training started at 120 days of age. After the first successful collection, semen was collected manually every 2 weeks by a single technician. At the semen collection, gelatinous material was separated from the liquid portion by filtration through double gauze. The volume of the liquid portion, the weight of gelatinous material, pH, and the sperm concentration, motility and morphology were examined in each ejaculate within 90 min after collection. Sperm concentration was determined using a haemocytometer, and the total number of spermatozoa per ejaculate was calculated by multiplication of sperm concentration and volume of the liquid portion. The spermatozoa were evaluated for their motility and morphology according to the procedures described previously¹⁶⁾. In brief, motility estimates were made in a small drop of semen under a cover slide using a bright-field microscope equipped with a heated stage (37°C). An aliquot of semen was fixed with an equal volume of 0.25 % (V/V) glutaraldehyde in phosphate-buffered saline, smeared on a glass slide, and stained in a phosphate-buffered solution of Giemsa stain (Merck, Darmstadt, Germany). Approximately 200 spermatozoa were counted at random by light microscopy to determine the percentage of morphologically abnormal spermatozoa.

Storage of semen at 4°C and -196°C: TCG solution¹⁴⁾, composed of Tris (hydroxymethyl)-aminomethane (1.21 g/90 ml), citric acid (0.60 g/90 ml), glucose (3.00 g/90 ml) and penicillin (90,000 I.U./90 ml), was used as a basic diluent. The semen collected from Group B was centrifuged for 10 min at 500 × g and the resulting sperm pellets were resuspended in TCG solution containing egg yolk (10% V/V) and catalase (15,000 Sigma units/100 ml, Sigma Chemical Co., St. Louis, USA) to give a concentration of approximately 2×10^8 cells/ml. Sperm suspensions were cooled to 4°C over 4h and stored for 5 or 10 days. In the case of freezing spermatozoa, sperm pellets obtained after centrifugation were resuspended in TCG solution containing egg yolk (20% V/V) to give a concentration of approximately 4×10^8 cells/ml, and then frozen into pellets of 0.2 ml on the surface of dried ice¹⁹⁾ following an additional dilution with an equal volume of the above-mentioned diluent containing glycerol (2% V/V)⁵⁾. After being stored in liquid nitrogen for more than 2 days, 2 frozen pellets were thawed in 1 ml of Beltsville thawing solution prewarmed at 40°C²²⁾.

A portion of the 4°C-stored or frozen-thawed sample was shaken at 37°C for 15, 30 and 60 min, after which the percentages of progressively motile spermatozoa were estimated and the maximum percentage was recorded. To determine the percentage of live spermatozoa with normal acrosomes, the remaining sample was diluted with an equal volume of TCG solution containing 1% trypan blue, incubated at 37°C for 15 min, smeared on glass slides and air-dried. The slides were rinsed in water, blotted and fixed in Orth solution at room temperature for 45 min. After fixation, they were rinsed in water, blotted and stained in buffered Giemsa solution at room temperature for 90 min. The stained smears were rinsed in water, air-dried and mounted. Approximately 200 spermatozoa for each preparation were counted at random with light microscope at

1000 ×.

Analysis of results: Data were analyzed using Duncan's multiple range test²³⁾.

Results

In 2 of the boars examined, detachment of the penis from the prepuce was found as early as 45 to 49 days of age and in the rests by 64 days. The average age of detachment was 57 days.

The first ejaculate could be collected from boars of Group A at 73 to 78 days of age. Observations of the appearance of semen during ejaculation revealed that the semen from Group A was much less opaque throughout the whole ejaculatory process, although opaqueness of the semen increased with the passage of age. The semen collected from Group B was more opaque than that from Group A. However,

Fig. 1. Volume of the liquid portion, weight of gelatinous material and pH of the ejaculate collected from Meishan boars at various ages in Group A (●) and B (▲). Values are means of 5-7 ejaculates. "n" months of age indicates a period between "30 × n" and "30 × (n + 1) - 1" days of age.

the sperm-rich fraction was not clearly distinguished from the sperm-poor fraction until 7 months of age.

As shown in Fig. 1, at 2 months of age, the volume of the liquid portion and the weight of gelatinous material were 23 ml and 9 g, respectively, and thereafter increased significantly until 11 and 10 months, respectively. The pH value of the ejaculates gradually declined until 7 months of age, followed by only minor fluctuations.

As shown in Fig. 2, although the total number of spermatozoa per ejaculate varied between individuals, it increased slowly from 2 (6.5×10^6 to 3.4×10^9 cells) to 4 months of age

Fig. 2. Total number of spermatozoa, sperm concentration, and percentages of progressively motile spermatozoa and of morphologically abnormal spermatozoa in ejaculates collected from Meishan boars at various ages in Group A (●) and B (▲). Values are means of 5-7 ejaculates.

^aSee footnote in Fig. 1.

Table 1. 4°C-storage of spermatozoa ejaculated from Meishan boars (Group B) at various ages

Age in months ^a	No. of ejaculates examined	At 4°C for			
		5 days		10 days	
		Progressively motile sperm (%)	Live sperm with normal acrosomes(%)	Progressively motile sperm (%)	Live sperm with normal acrosomes(%)
5	4	64±8	35±3 ^d	43±5	23±3 ^c
6	5	73±6	41±5 ^{cd}	55±2	22±2 ^c
7	6	72±5	42±3 ^{cd}	62±3	20±4 ^c
8	3	78±4	51±6 ^{bcd}	43±9	20±2 ^c
9	3	77±3	61±3 ^b	60±8	43±4 ^b
10	6	79±2	58±4 ^{bc}	58±4	38±4 ^b
11	5	72±4	56±2 ^{bc}	48±4	38±3 ^b

Values are means±SEM.

^aSee footnote in Fig. 1.

^{bcd} Values within the same columne with different superscript letters differ significantly, $P<0.05$.

(2.4×10^9 to 1.5×10^{10} cells) and then rapidly until 5 months (1.1 to 3.1×10^{10} cells). After 5 months of age, it showed a steady but slow increase until 9 months (2.1 to 4.4×10^{10} cells). The increase in the sperm concentration occurred until 5 months of age, followed by no significant changes. The percentage of progressively motile spermatozoa was 35% at 2 months of age, and then it increased rapidly to 83% at 3 months. Morphologically abnormal spermatozoa, which were mainly abnormal heads, were contained at a high level (33%) in the ejaculates collected at 2 months of age. At 3 months of age, however, the percentage reduced to 7%, and thereafter stayed between 3 and 12%.

As shown in Table 1, the percentage of progressively motile spermatozoa after 4°C-storage was not significantly affected by the age of the boars at semen collection. However, the percentages of live spermatozoa with normal acrosomes after storage for 5 and 10 days were significantly higher in samples collected at more than 9 months of age.

As shown in Table 2, the percentages of progressively motile spermatozoa and of live spermatozoa with normal acrosomes after frozen storage were higher in samples collected at

Table 2. Frozen storage of spermatozoa ejaculated from Meishan boars (Group B) at various ages

Age in months ^a	No. of ejaculates examined	Progressively motile sperm (%)	Live sperm with normal acrosomes(%)
7	3	25±9 ^c	16±0 ^c
8	3	47±3 ^b	36±2 ^b
9	3	50±3 ^b	40±1 ^b
10-11	4	50±3 ^b	38±2 ^b

Values are means±SEM.

^aSee footnote in Fig. 1.

^{bc} Values within the same columne with different superscript letters differ significantly, $P<0.05$.

more than 8 months of age.

Discussion

In all of the boars examined, the penis was detached from the prepuce by 64 days of age. The first ejaculate could be collected from boars of Group A at 73 to 78 days of age. These results suggest that Meishan boars acquire ejaculatory ability soon after their penis is freed from the prepuce, at approximately 2 months of age. According to previous studies on boars of Yorkshire^{4,10)}, Berkshire²⁰⁾, Large White²¹⁾, Poland China²¹⁾, Duroc⁴⁾, cross-bred Yorkshire-Duroc-Hampshire¹⁷⁾ and cross-

bred Yorkshire-Hampshire-Duroc breeds²⁾, penile detachment from the prepuce and first collection of ejaculates occur at 4 to 5 and 5 to 6 months, respectively, which are remarkably later than those in Meishan boars.

The volume of the liquid portion of the ejaculates and the weight of gelatinous material increased significantly until 11 and 10 months of age, respectively. This indicates that the secretory activity of the accessory genital glands is enhanced with the passage of age. Indeed, the seminal vesicle continues to grow until 7 to 11 months of age⁸⁾.

Ito *et al.*¹¹⁾ reported that fresh semen containing more than 4.0×10^9 cells was necessary to achieve reasonable fertility for artificial insemination. According to Johnson *et al.*^{12,13)}, when diluted semen containing 3.0×10^9 spermatozoa were inseminated into sows on the same day of the collection, farrowing rates, total number of piglets and number of live piglets per litter obtained were satisfactory. Based on these reports, it is necessary to inseminate at least 3.0×10^9 spermatozoa into the female for the purpose of high fertility and large litter size. In the present study, the total number of spermatozoa per ejaculate did not constantly exceed 3.0×10^9 cells until 5 months of age, although sperm motility and morphology reached a definitive level as early as 3 months. We have reported that the weight of the testis and epididymis and the diameter of the seminiferous tubules increased rapidly until 5 to 6 months of age^{6,7)}. These findings suggest that Meishan boars should not be used in service until after 6 months of age. In fact, Meishan boars begin to be used at 6 to 8 months of age in breeding farms in China^{3,24)}, where most of the services are performed by means of artificial insemination using diluted whole semen on the same day of collection or after short-term storage for less than 2 days at 13 to 20°C¹⁵⁾. However, the results obtained in this study suggest that when relatively long-term storage of diluted semen at 4°C or

frozen storage is needed, boars older than 9 months should be used as donors.

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種々の月齢で採取した梅山豚精液の性状

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雄梅山豚における生殖機能の発達過程を明らかにする目的で、射精能力の発達および月齢に伴う精液性状の変化について検討した。包皮からの陰茎遊離は、検査した12頭のすべてにおいて64日齢までに確認された。手掌圧迫法により初めて精液が採取できたのは73～78日齢であった。採取された精液の液体部量、膠様物重量、1射精当りの総精子数および精子濃度はそれぞれ11カ月齢、10カ月齢、9カ月齢および5カ月齢まで有意に増加したが、前進精子率および奇形精子率については、3カ月齢で採取した精液で成豚の精液に匹敵する成績が得られた。他方、5日間および10日間の4℃保存後の試料における正常な頭帽を持つ生存精子の割合は、9カ月齢以上の雄から採取した精液を用いた場合に有意に高く、また、凍結保存後の試料での前進精子率および正常な頭帽を持つ生存精子の割合は8カ月齢以上の雄の精液において良好であった。以上のように、雄梅山豚は約2カ月齢で射精能力を獲得するが、繁殖用種雄として供用するのは6カ月齢以降にすべきと考えられる。また、精液を4℃あるいは-196℃で保存する場合には、9カ月齢以上の雄から採取した精液を用いることが望ましいと思われる。

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