Application of Artificial Insemination in Selective Breeding of Native Beef Cattle in Wenzhou

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Abstract **[Objective]** The paper was to improve the quality and growth rate of native yellow cattle in Wenzhou. **[Method]** Three groups of cattle were subjected to artificial insemination using frozen semen from Wagyu bull. The groups consisted of 20 native yellow cattle, 20 Angus cattle, and 20 Luxi yellow cattle. The heifers were subsequently evaluated for body size and body weight, and underwent treatment with estrus synchronization, artificial insemination, and early pregnancy diagnosis. **[Result]** The mean body size and body weight of native yellow cattle were found to be significantly lower than those of Angus cattle and Luxi yellow cattle ($P<0.05$). The estrus synchronization rate and mating rate of native yellow cattle were both 100%, while the corresponding rates for Angus cattle and Luxi yellow cattle were 90% and 95%, respectively. The ultrasound examination conducted on the 32[nd] day following the mating revealed a distinct image of gestation sac, which, when considered alongside the findings of the rectal examination, may be indicative of pregnancy. The conception rates were 75%, 72%, and 74% for native yellow cattle, Angus cattle, and Luxi yellow cattle, respectively. **[Conclusion]** The reproductive performance of native beef cattle is relatively superior.

Keywords Native cattle in Wenzhou; Angus cattle; Luxi yellow cattle; Artificial insemination; Estrus synchronization; Fertilization rate

Since ancient times, Wenzhou local cattle have been primarily utilized for agricultural purposes, including plowing and transportation of supplies. One distinctive feature is a high shoulder peak, which differs from the shoulder peak of Wenling humped cattle. The shoulder peak of Wenzhou cattle is more rounded in shape. As mechanization and automation advanced, the local cattle population underwent a gradual shift from a primarily service-oriented to a primarily meat-oriented species. The dietary concepts and preferences of the general public continue to evolve, and the quality standards for beef are becoming increasingly demanding. This has led to the development of medium- and high-grade beef cattle, which are being bred on a larger scale. The foundation of large-scale production of medium- and high-grade beef cattle is breed breeding[1]. The key to effective breed breeding is artificial insemination technology, which offers several advantages, including improved reproductive efficiency, accelerated genetic improvement, prevention of disease spread, and facilitation of large-scale breeding and reproduction management[2]. The technology of artificial insemination in beef cattle[3] is based on three main operations: estrus identification, frozen semen preparation, and insemination[4]. Estrus identification is the process of observing the external performance of the female cattle in heat in order to determine which females are in heat and thus eligible for artificial insemination. This step is crucial for the success of the breeding program. The application of artificial insemination technology has the potential to overcome the limitations of time and space associated with the use of good breeding bulls for the purpose of breed crossbreeding improvement. This has the capacity to enhance the growth rate, body shape and meat quality of native beef cattle, which is of significant importance.

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1 Materials

1.1 Test site and animals The Wagyu frozen semen (breeding bull No.: 37314102) was procured from Shandong OX Livestock Breeding Co., Ltd. The test was conducted in Taishun County, Wenzhou City, Zhejiang Province, China. The females were of the following breeds: native yellow cattle, Angus cattle, Luxi yellow cattle. Each breed was represented by 20 individuals, sourced from Rongfa Agricultural Professional Cooperative and Maolikan Breeding Co., Ltd., both located in Taishun County, Zhejiang Province, China.

1.2 Main instruments The following instruments were procured from Zhejiang Lobang Import and Export Co., Ltd.: a liquid nitrogen tank (Dongya YDS-35-125), a microscope (display screen) (Motic, M150), a cattle backfat detection and pregnancy diagnosis instrument. The aforementioned instruments, equipment, and consumables were provided by the Laboratory of Livestock Reproduction and Breeding at the Wenzhou Institute of Agricultural Sciences.

2 Methods

2.1 Experimental design A total of 20 individuals of each breed of native yellow cattle, Angus cattle, Luxi yellow cattle were selected as bred recipient females from Rongfa Agricultural Professional Cooperative and Maolikan Breeding Co., Ltd. for breeding purposes. Subsequently, the selected females were subjected to a series of measurements, including body size and weight, as well as estrus synchronization, artificial insemination, and early pregnancy diagnosis.

2.2 Estrus synchronization The estrus synchronization treatment procedure was as follows: On the morning of day 0, the cloprostenol sodium (PG) solution was injected at 10:00. The solution was prepared by dissolving and diluting 0.5 mg of PG in 5 mL of normal saline. The estrus cattle were observed on the 1st and 2nd day of the study and were then mated at the conclusion of the estrous cycle. For cattle that were not in estrus, 0.5 mg of PG was administered via injection, diluted with 5 mL of normal saline, on the 11th day. The cattle that were in estrus were observed on the 12th day and subsequently mated.

2.3 Sperm motility test Sperm motility is the percentage of forward-moving sperms out of the total number of sperms at 37 ℃[5]. Sperm motility (%) = (Number of forward–moving sperms/Total number of sperms) ×100%. The fine tube of frozen sperm should be removed from the liquid nitrogen tank and promptly placed into a 38 ℃ thermos. It should be noted that other frozen sperm can not be removed from liquid nitrogen. The sealed end of the frozen semen sample was cut open and placed into a 0.5 mL centrifuge tube. The cotton plug at the end of the tube was removed, allowing the semen to flow into the centrifuge tube. A pipette was used to aspirate 30 μL of the sample onto a sterilized slide, and the coverslip was covered. The microscope magnification was adjusted to 200×, and the percentage of sperms moving in a straight line was observed through the display to record the motility of frozen sperms in fine tubes[5].

2.4 Artificial insemination The cattle mating rate is defined as the percentage of the number of successfully completed mating in a given time period to the total number of specific cattle selected in the same time period. It can be expressed as follows: Cattle mating rate (%) = (Number of cattle mated/Total number of cattle) × 100%. It is recommended that frozen semen should be thawed in fine tubes and that the recipient cattle should be mated within 3 h of the end of estrus. Additionally, pregnant cattle must be provided with additional nutrition to support fetal development and growth.

2.5 Diagnosis of early pregnancy Following the breeding of cows, the completion of the sperm-egg union initiated the migration of the embryo from the oviductal section toward the uterus. This process of attachment was gradually completed. On the 32nd day following the breeding, the uterine body and uterine horn area of the cow could be palpated by rectal examination in order to ascertain the size and condition of the two uterine horns. The next step was to locate the finger belly along the tip of the uterine horn in order to identify one side of the ovary. The size and elasticity of the ovarian volume should then be assessed in order to determine whether the corpus luteum or follicle was present. Once this had been established, the ultrasound image can be combined with the findings to determine the pregnancy status. This allowed for an accurate determination of the cow's pregnancy.

2.6 Statistical analysis of data The data were analyzed using Excel software, and the results were expressed as mean ± standard deviation.

3 Results and Analysis

3.1 Detection of sperm motility of frozen semen in fine tubes The index parameter testing of fine tube frozen semen was conducted in accordance with the *National Standard of the People's Republic of China for Cattle Frozen Semen* (GB4143-2008). The key indicators, including frozen semen appearance, dosage of the agent, and sperm motility, were found to be higher than the national standard, as evidenced by the results presented in Tab.1.

3.2 Body size, body weight, estrus synchronization and mating effects of native yellow cattle and control recipient cows Native yellow cattle exhibited the lowest body weight and size, which were significantly lower than those of Angus cattle and Luxi yellow cattle ($P<0.05$, Tab. 2). Native yellow cattle exhibited the highest mating rate of 100%, while Angus cattle exhibited the lowest at 90%, as shown in Tab.3.

3.3 Pregnancy diagnosis of different native yellow cattle The conception rates for native yellow cattle, Angus cattle, and Luxi yellow cattle were 75%, 72%, and 74%, respectively, as shown in Tab.4.

Tab.1 Examination results of frozen sperm in fine tubes

Item	Examination results	National standard GB4143–2008
Fine tube wall and end seals	Up to standard	No cracks in the fine tube wall and tight sealing at both ends
Dosage//mL	0.24	0.25
Sperm motility//%	39±2	≥35

Tab.2 Body size and weight

Group	Withers heigh// cm	Height at hip cross// cm	Body length// cm	Straight length// cm	Chest circumference// cm	Abdominal circumference cm	Circumference of cannon bone// cm	Ischial end width// cm	Body weight// kg
Native yellow cattle	108.0 a±5.5	113.0 a±5.3	126.0 a±5.6	122.0 a±6.0	158.0 a±5.8	192.0 a±5.4	15.0 a±2.5	15.0 a±3.3	322.5 a±9.8
Angus cattle	135.0 b±2.5	136.0 b±2.4	144.0 b±2.9	137.0 b±2.8	185.0 b±3.1	214.0 b±3.3	19.0 b±1.2	18.0 b±1.9	471.0 b±10.2
Luxi yellow cattle	130.0 c±4.0	129.0 c±4.5	154.0 c±4.6	142.0 c±4.2	171.0 c±5.0	207.0 c±4.9	17.0 c±1.90	17.0 c±2.1	425.0 c±8.8

Note: Different lowercase letters in the same column indicates a statistically significant difference at the 0.05 level.

Tab.3 Estrus synchronization and mating of cattle

Group	Number of cattle injected with PG on day 0	Number of cattle in heat on the 1st and 2nd day	Number of mating cattle	Number of cattle injected with PG on the 11th day	Number of cattle in heat on the 12th and 13th day	Number of mating cattle	Total estrus rate// %	Total mating rate// %
Native yellow cattle	20	10	10	10	10	10	100	100
Angus cattle	20	9	9	11	9	9	90	90
Luxi yellow cattle	20	10	10	10	9	9	95	95

Tab.4 Fertility rate of recipient cattle

Group	Number of mating cattle	Number of pregnant cattle in pregnancy diagnosis	Conception rate in pregnancy diagnosis// %
Native yellow cattle	20	15	75
Angus cattle	18	13	72
Luxi yellow cattle	19	14	74

Fig.1 Ultrasound pregnancy image on the 32nd day after mating (with visible gestational sac showing pregnancy)

Fig.1 depicts ultrasound images of pregnancies at day 32 post-mating.

4 Discussion and Conclusions

The timing and operation technique of insemination represent a crucial aspect in the improvement of conception rates[6], while nutrition also plays a pivotal role in enhancing reproductive efficiency. The supplementation of n-6/n-3 polyunsaturated fatty acids (PUFA) to the diet of recipient cows has been demonstrated to inhibit the endometrial synthesis of prostaglandin F2α (PGF2α), thereby promoting the recognition of pregnancy and embryo attachment[7]. The local climate and feeding conditions have led to the evolution of native beef cattle, which have adapted to these conditions over time. This adaptation has resulted in a higher conception rate than that observed in the control group. The application of artificial insemination techniques can facilitate the acceleration of crossbreeding improvement. The progeny of Angus cattle crossbreeding exhibit accelerated growth, rapid weight gain, high meat yield, and superior meat quality when compared with native yellow cattle[8]. Following the crossbreeding of Heimaohe cattle with Yanbian cattle, the F_2 generation exhibited significantly higher body weight, body height, body length, chest circumference, tube circumference, and other indice of both bulls and cows in comparison to the F_1 generation. This indicates that the crossbreeding potential of this breed is greater[9-10]. The carcass weight and net meat weight of cattle in F_1 and F_2 generations at different ages were found to be greater than those of Dabieshan cattle in the crossbreeding improvement of Dabieshan cattle by Red Angus cattle[11]. The study of meat quality in West Hybrid Cattle, including the F_1 generation of Angus and West Hybrid Cattle crossbreds and the F_2 generation of Angus and West Hybrid Cattle crossbreds, revealed that the beef of Angus and West Hybrid crossbred progeny was more tender and contained more intermuscular fat, with the potential to produce high-end beef[12]. Xinjiang Brown (BD) cattle were economically crossed with meat breeds-South Devon cattle (ND), Red Angus (AG), and meat Simmental cattle (XM)-to determine if they could demonstrate significant hybrid dominance and a favorable fit. The results demonstrated that the mean birth weight of the ND ×BD group was significantly higher than that of the other two groups, while that of the AG×BD group was the lowest. The weight of the AG×BD group exhibited a rapid increase with the advancement of months of age, indicating robust growth in the early stages of development. However, with the continuous increase in months of age, the weights of the AG×BD group and the other two groups gradually approached each other, starting from the age of 6 months. At 12 months of age, the chest width of the AG×BD group was found to be significantly greater than that of the other two groups, although no differences were observed in other indices. The optimal crossbreeding combination was identified as the ND×BD group, followed by the AG×BD group and finally the XM×BD group[13-14]. The promotion of muscle growth in cattle has been observed to result in missense mutations in the inhibin gene, which in turn has been linked to the development of myoblast hyperplasia and myofiber hypertrophy. This phenomenon has been shown to result in a notable increase in muscle mass and the emergence of a dual-muscle phenotype[15].

The Wenzhou native beef cattle population exhibits considerable variation in size, with individuals of both small and large stature. These animals exhibit a relatively slow growth rate, and a characteristic that is associated with their relatively tender meat. Additionally, they mature at an early age and demonstrate high levels of stress resistance. Furthermore, the reproductive performance of these cattle is noteworthy, with a 100% mating rate and an artificial insemination fertilization rate of 75%. The Angus cattle are distinguished by their taller and heavier stature, with a 90% mating rate and a 72% conception rate. In comparison, the Luxi yellow cattle exhibit a longer diagonal and straight length, with a 95% mating rate and a 74% conception rate. Consequently, the reproductive performance of native beef cattle is relatively high. Cattle artificial insemination represents a significant technological advancement in the field of cattle reproduction and genetic improvement. Its development has become a crucial aspect in the advancement of cattle breeding. The limitations of time and space for breeding bulls can be overcome, and the spread of epidemics can be reduced, thus allowing for further breakthroughs in the improvement of cattle reproductive efficiency, genetic characteristics, and production levels. As technology continues to be perfected, these improvements can be expected to continue.

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