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Investigation of Reproductive Traits and a Computer-Assisted Single Artificial Insemination Procedure in Mares in Subtropical Taiwan

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Abstract: The objective of this study was to report the reproductive traits of mares by ultrasonography and hormone analyses in subtropical Taiwan and the successful artificial breeding of mares using a self-developed computer-assisted single insemination technique. A previously developed computer-assisted image analysis technique at our reproduction centre was employed to determine the optimal time for artificial insemination (AI). In the 8 mares that underwent this AI procedure, ovulations all occurred within 12 h post-insemination and 5 mares were impregnated (62.5%). The combined results of ultrasonic examination and hormone analyses showed that the mares in Taiwan were not only possible defined seasonal breeders, but also polyoestrous breeders that could be bred throughout the year. It is suggested that mares could be injected with only 1 dose of 500×10^6 total spermatozoa into the uterine body at the time determined by this developed programme to obtain a satisfactory pregnancy rate.

Key Words: Reproductive traits, computer-assisted, artificial insemination, mare

Artificial insemination (AI) has become a mature practice for equine reproduction throughout Europe and North America. To achieve a maximal pregnancy rate with frozen-thawed semen, it is necessary to inseminate mares in a period between 12 h pre- and 6 h post-ovulation (1,2); however, accurate prediction of ovulation remains a big challenge. In addition, the quality reduction in the cryopreserved semen usually means that the mares have to be inseminated twice or more at a minimal number of $150\text{-}300 \times 10^6$ total spermatozoa per cycle (1,3), rendering the breeding cost variable depending on the number of AI procedures performed. In order to maintain a satisfactory fertility rate with the number of AI procedures kept at a minimum to reduce the cost, we report here the first successful artificial breeding of mares in Taiwan using a self-developed computer-assisted single insemination procedure. Hormonal analyses and the oestrous cycles of the mares by ultrasonography were studied to investigate any reproductive traits that might be characteristic for the subtropical country Taiwan.

For the study of reproductive traits, a total of 109 complete oestrous cycles of normal mares aged 3-20 years were investigated by ultrasonography. Serum estradiol and progesterone concentrations were determined by chemiluminescence immunoassay (Vitros Eci Immunodiagnostic System). Eight mixed breed mares aged 4-14 years and weighing 450-550 kg, exhibiting regular oestrous cycles year round, were prepared for this AI procedure. Uterine swabs of the mares in dioestrus for bacterial culture and cytological examination proved to be free of endometritis. A computer-assisted image analysis technique previously developed at our reproduction centre was employed to determine the optimal time for AI (4). Briefly, the pixel intensity of the follicular wall was quantified as a slope value, which serves as an indicator to identify follicles that were to ovulate in 24 h. Once the follicle and oedematous uterine endometrium on the first day of oestrous were noted, the changes in dominant follicles were monitored daily with a real-time B-mode linear assay ultrasound scanner, equipped with a 7.5 MHz transrectal probe.

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Ultrasonographic images were recorded consecutively for computer-assisted analysis. The imminence of ovulation within 24 h was determined when the quantified slope value from the pixel changes of the follicular wall was 19.0 (Figure 1). AI into the uterine body with 1 dose of frozen-thawed semen containing 500×10^6 spermatozoa from a Hanoverian stallion was immediately performed once the preovulatory follicles of the mares were recognised. The pregnancy was subsequently confirmed on day 16 after the AI procedure by ultrasonography.

In the current study, the younger mares (3-9 years) showed a significantly shorter oestrous cycle duration than mares that were older than 10 years (22.9 ± 1.6 days vs. 27.9 ± 2.2 days, $P < 0.05$). All mares developed

ovulatory follicles ≥ 30 mm in diameter. The concentrations of serum oestradiol increased progressively from D -5 (D 0 = ovulation, D -1 = the day prior to ovulation) to D -1 (25.3 ± 1.5 vs. 42.6 ± 2.1 pg/ml), maintained at 28.7 ± 1.5 pg/ml on D 0, and then decreased markedly to 18.7 ± 1.3 pg/ml on D 3. On the other hand, the progesterone concentrations were significantly higher on D 3 (5.4 ± 0.4 ng/ml) and the following luteal phase on D 8 (10.1 ± 1.5 ng/ml) than those in oestrous (< 1 ng/ml, $P < 0.01$). The combined results of ultrasonic examination and hormone analyses indicated that most of the young mares in Taiwan can show oestrous cycles accompanied by ovulations throughout the year (Figure 2). Of the 8 mares that underwent this AI procedure, ovulations all occurred within 12 h post-insemination and 5 mares were impregnated (62.5%) in different months including one in December when very few mares naturally conceived. Five healthy foals were born subsequently.

It is known that physiological adaptations by mares were possible and that their reproductive behaviour changes were due to environmental factors, such as day length, food intake, and temperature (5). A previous observational study documenting the reproductive performance of crossbred mares in middle Taiwan indicated that mares exhibited oestrous cycles all year round, with more than 80% of them in heat during March to October (6). The results of this study indicated that the mares in Taiwan were not only possible defined seasonal breeders but also polyoestrous breeders that could be bred throughout the year. This finding was in agreement with the previous observation of an extended acclimation by these animals in subtropical Taiwan (6). Clinically, 3000 IU of human chorionic gonadotropin (hCG) is routinely used to induce ovulation when at least one dominant follicle of ≥ 30 mm is detected after the beginning of oestrous. Ovulation usually occurs within 48 h in about 90% of mares that responded to hCG and about 75% of these happened within 25-48 h after hCG treatment (1). Although the change in the follicle size and shape and the ultrasonic echotextural characteristics of the follicular wall during the preovulatory period were commonly used to predict ovulation, there is no widely accepted effective indicator for accurate prediction of ovulation (7,8). This quantitative echotexture analysis we developed could be used for close prediction (within 1 day) of impending ovulation and was successfully

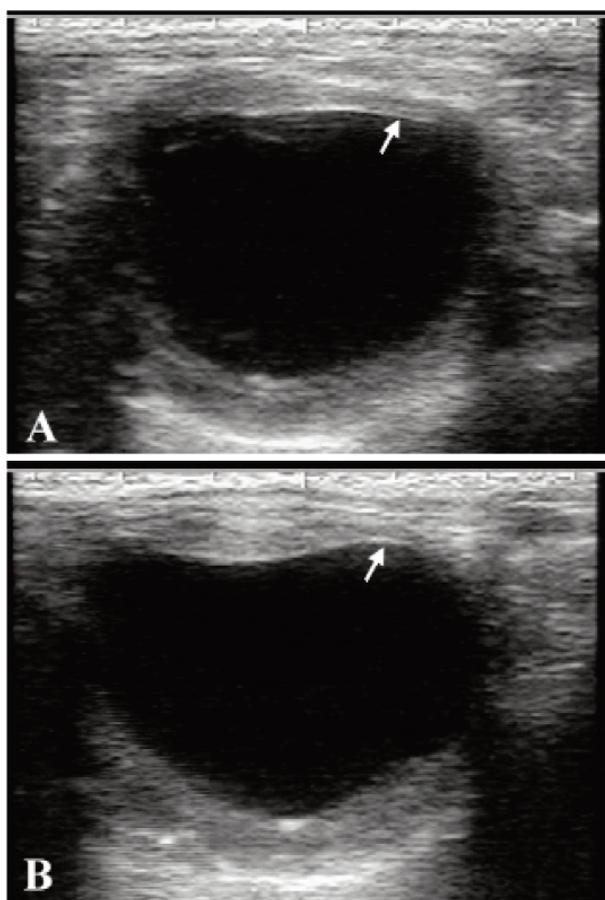


Figure 1. The echotextural changes of the follicular wall at the 2 o'clock position (arrows) were quantified as a slope value by pixel intensity analysis. The dominant follicle (A) measuring at 4.0×3.2 cm and 11.8 for the slope value on D-2 is compared with the preovulatory follicle (B) of 4.2×3.1 cm in size with a slope value of 20.7 on D-1. The distance between the scale marks at the top of the picture is 10 mm.

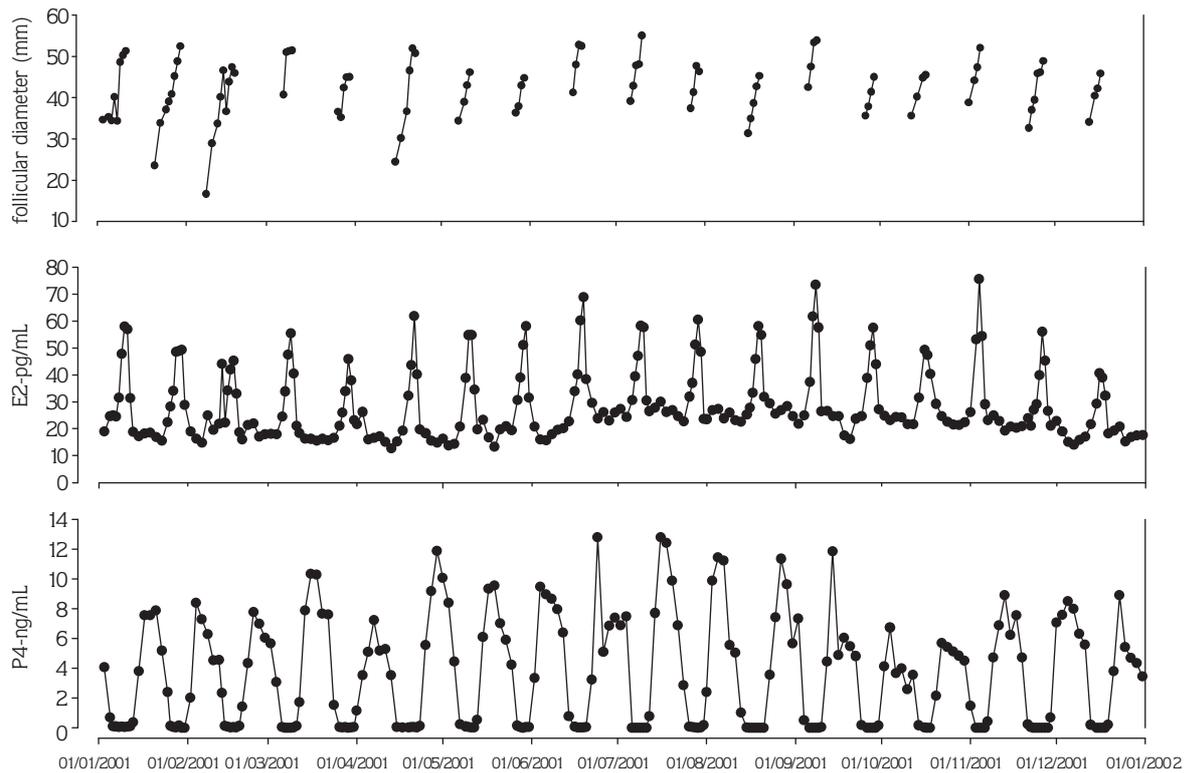


Figure 2. A continuous record of ultrasonic examination and hormone analyses in a young mare throughout the year. A total of 18 complete oestrous cycles accompanied by ovulations and cyclic changes of plasma progesterone and oestradiol concentrations represented the characteristic year-round breed type of the mares in subtropical Taiwan.

incorporated into this AI procedure. The choice of insemination site was also critical in developing the procedure, since insemination into the uterine body provided higher pregnancy rates than insemination deep into the uterine horn (2,9). Overall, our results suggested that, with the assistance of the developed computer image analysis to predict ovulation, mares could be injected with only 1 dose of 500×10^6 total spermatozoa into the uterine body at the time determined by our developed programme to obtain a preliminarily satisfactory pregnancy rate (62.5%) in comparison with previous reports of 22%-60% per cycle with frozen semen

(2,3,10). A further study is therefore necessary to amplify the sample size for a statistically more precise result, so that this AI procedure could become a routine practice for equine reproduction.

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