Jap. J. vet. Res., **22**, 61~71 (1974)

STUDIES ON PREGNANCY DIAGNOSIS IN DOMESTIC ANIMALS BY AN ULTRASONIC DOPPLER METHOD

I PREGNANCY DIAGNOSIS IN THE PIG AND FETAL HEART RATE CHANGES DURING PREGNANCY*1

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(Received for publication, January 11, 1974)

A portable ultrasonic fetal heart detector, Heart-tone Model USD-I (Aloka Co. Ltd., Tokyo), with a 2.25 MHz probe was used for a study of pregnancy diagnosis in swine.

The animals to be examined were placed lying on the pen floor with neither fixation nor anesthesia. The probe was gently applied on the skin of the lower abdomen or the flank. Ultrasonic Doppler signals based on the fetal circulation were heard by the operator and at the same time recorded on magnetic tapes to aid fetal heart rate calculation. The maternal circulation was easily distinguishable from the fetal circulation by the considerably slower rhythm of the former. The accuracy of diagnosis was based on delivery.

A total of 83 experiments were carried out on 70 sows of 4 different breeds from 22 days post-coitum to the full term. Before 30 days post-coitum, the accuracy rate for pregnancy diagnosis was not satisfactory (58.3%). However, it became higher during the period from 30 to 39 days (81.8%). After 40 days of pregnancy, the accuracy rate of diagnosis reached 100 percent. The earliest detection of pregnancy by this method was in a case of 26 days after copulation.

A highly significant negative correlation was observed between the fetal heart rate and the fetal age. The correlation coefficient was -0.87 ($P<0.01$). The fetal heart rate became slower in a negative linear regression as gestation progressed; $Y=252.6-0.572X$ (X=Fetal age, Y=Fetal heart rate).

INTRODUCTION

Pregnancy diagnosis in domestic animals has become increasingly important

*1 Partially supported by a grant from the Scientific Research Fund of the Ministry of Education (No. 86601, 1970)

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to the livestock breeder and the veterinary surgeon. In the swine species, several methods for pregnancy checking have hitherto been described. They include chemical or biological tests of urinary estrogens[6,14,35,40], a progesterone assay in serum[34], a serum protein analysis[3], a rectal palpation test[17,24], an X-ray method[43], a test for the specific gravity measurement of cervical mucus[32], a vaginal biopsy method[7,16,25,41], etc. No satisfactory test, however, has yet been made available for diagnosing pregnancy in swine, because of various unfavorable factors, such as difficulties in the fixing of animals, the difficulty of collecting materials, the long period of time involved, the low accuracy rate or inapplicability in the early gestational stages.

Recently, a new method for pregnancy diagnosis using ultrasonic waves has been developed. In human obstetrics, the ultrasonic method is now widely used as one of routine diagnostic means in detecting abnormal pregnancies[38], monitoring parturition courses[37], etc., in addition to the diagnosis of normal pregnancy[1]. In the veterinary field, since LINDAHL (1966) first succeeded in diagnosing pregnancy of ewes by an ultrasonic pulse method (A-scope), it has been reported by several investigators that ultrasonic procedures (pulse or Doppler method) are applicable to detecting pregnancy in various animals[2,4,5,8~11,15,18,23,27~30,42]. Since 1968, the present authors (KAWATA, TOO & FUKUI) have been studying pregnancy diagnosis of several kinds of mammals such as the pig, cow, dog, horse, cat, goat, sheep and rabbit by an ultrasonic Doppler method and they have made preliminary reports of parts of the results of these studies at some meetings[19~22,39].

The present paper deals with an accumulated result on the swine species.

MATERIALS AND METHODS

Experimental animals: Seventy sows of 4 different breeds were used (28 Landraces, 23 Large Whites, 17 Hampshires and 2 Yorkshires). The majority of these sows were kept in a farm of the Takikawa Animal Husbandry Experiment Station, where the reproductive records of the sows were well maintained. The animals were subjected to the experiment from 22 days post-coitum to the full term.

Ultrasonic Doppler equipments: A portable ultrasonic fetal heart detector, Heart-tone Model USD-I (Aloka Co. Ltd., Tokyo), 30×35×16 cm in size, 6.5 kg in weight, and a cylinder type probe, UST-2322 (Aloka Co. Ltd.), 3 cm in diameter, 9.8 cm in length, 80 g in weight, were used in this study. The apparatus works with an alternating current (100 V, 50 Hz). A continuous output ultrasonic wave of 2.25 MHz of low intensity (30 mW/cm^2) can be transmitted from the probe.

Experimental procedures: An animal to be examined is placed lying on

one side on the floor of the pen with neither fixation nor anesthesia. The probe is gently applied on the skin of the lower abdomen or the flank (fig. 1). Liquid paraffin is used as a coupling medium between the surface of the probe and the skin. Ultrasonic Doppler signals based on the fetal circulation are amplified and can be easily heard by the operator with the aid of a loudspeaker or earphones attached to the instrument. The sound can be conveyed to a tape-recorder, pen-recorder or oscillograph. Recording on magnetic tape is convenient for the calculation of the fetal heart rate when the rate is very fast. The block diagrams of the equipments used are illustrated in figure 2.

FIGURE 1 *Checking for pregnancy by the Doppler method on the lower abdomen of a Hampshire sow*

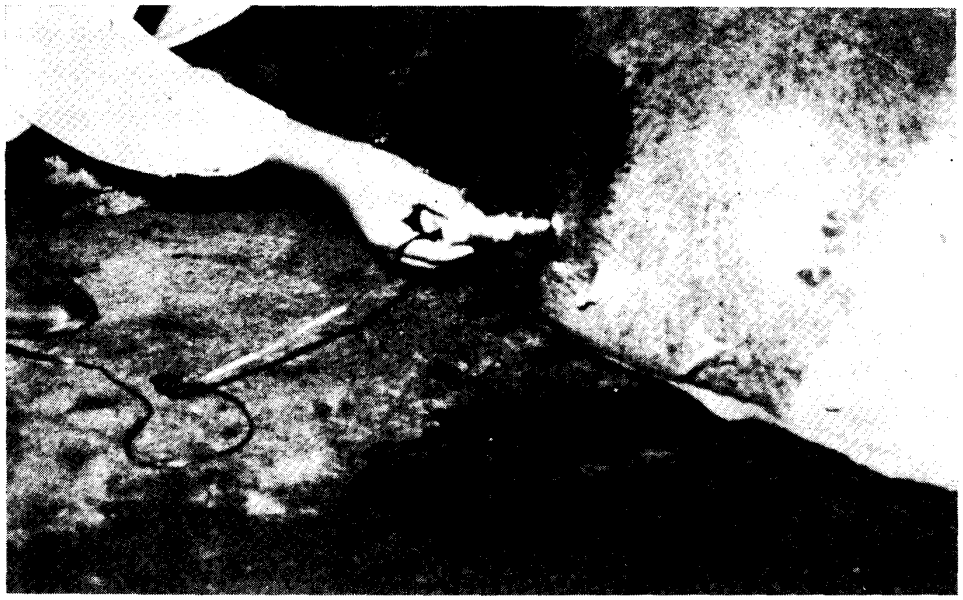
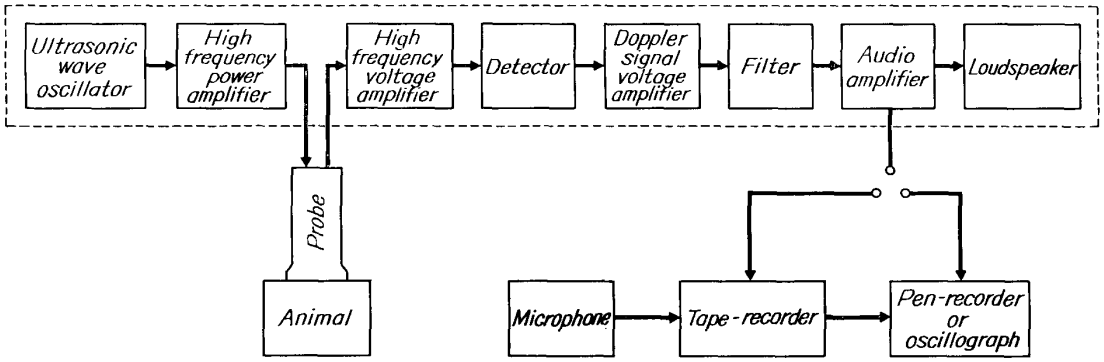


FIGURE 2 *Block diagrams of equipments used in the ultrasonic Doppler method. A square surrounded with dotted lines indicates an ultrasonic fetal heart detector.*



Criterion for diagnosing pregnancy: The fact that the Doppler signals reflected from the fetal heart produce a characteristic clapping sound with a regular and faster rhythm than that of the mother, is confirmed in the human by the simultaneous recording of the Doppler signal, fetal ECG and fetal PCG[31]. The present authors also made certain of the fact in some experiments by the simultaneous recording of the Doppler signal and fetal ECG in cattle and by means of the direct application of a Doppler probe on the pregnant uterus exposed by laparotomy in the cow, cat and dog. In addition, by laparotomy experiments of these species, it was verified that the Doppler signals originated from the umbilical artery caused a marked "whipping" or "swishing" sound with a rhythm synchronized with the fetal heart rate (unpublished data). Although, in the swine, no reports about such experiments have been published, it would be reasonable to think that Doppler signals of a continuous and rhythmical character and of a faster frequency than the maternal pulse rate may be attributable to the fetal circulatory system. Therefore, the present authors decided to use the occurrence of Doppler signals with the above mentioned characteristics as a criterion for diagnosing pregnancy in this study.

Results

1 Pregnancy diagnosis

A total of 83 experiments were carried out on 70 sows. The results for pregnancy diagnosis are shown in table 1. The accuracy of diagnosis was based

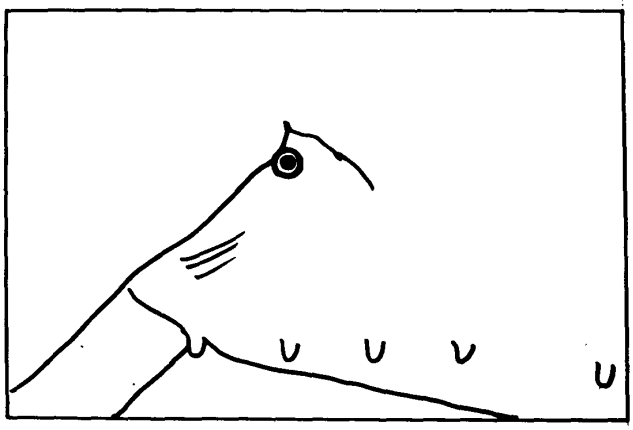
TABLE 1 *Accuracy rates of pregnancy diagnosis in various gestational stages*

DAYS IN GESTATION	NO. OF CASES EXAMINED	CORRECTLY DIAGNOSED*1	INCORRECTLY DIAGNOSED*2	ACCURACY RATE (%)
22~ 29	12	7 (3)	5	58.3
30~ 39	22	18 (3)	4	81.8
40~ 49	11	11 (1)	0	100.0
50~ 59	4	4	0	100.0
60~ 69	5	5	0	100.0
70~ 79	2	2	0	100.0
80~ 89	7	7	0	100.0
90~ 99	7	7	0	100.0
100~114	13	13	0	100.0
Total	83	74 (7)	9	89.2

*1 Sum of fetal Doppler signal positive and pregnant cases, plus fetal Doppler signal negative and non-pregnant cases (figures in parentheses)

*2 Fetal Doppler signal negative and pregnant cases only

FIGURE 3 *Diagram of preferable area of the flank for detecting early fetal life and pen-recorded fetal and maternal heart Doppler signals in a 32-day pregnant sow*



Fetal heart Doppler signals (arrows) 240/min

Maternal heart Doppler signals (arrows) 80/min

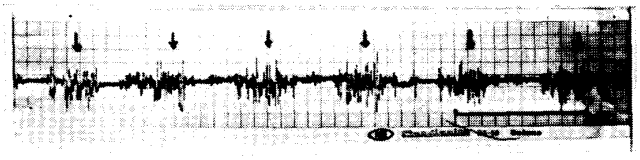
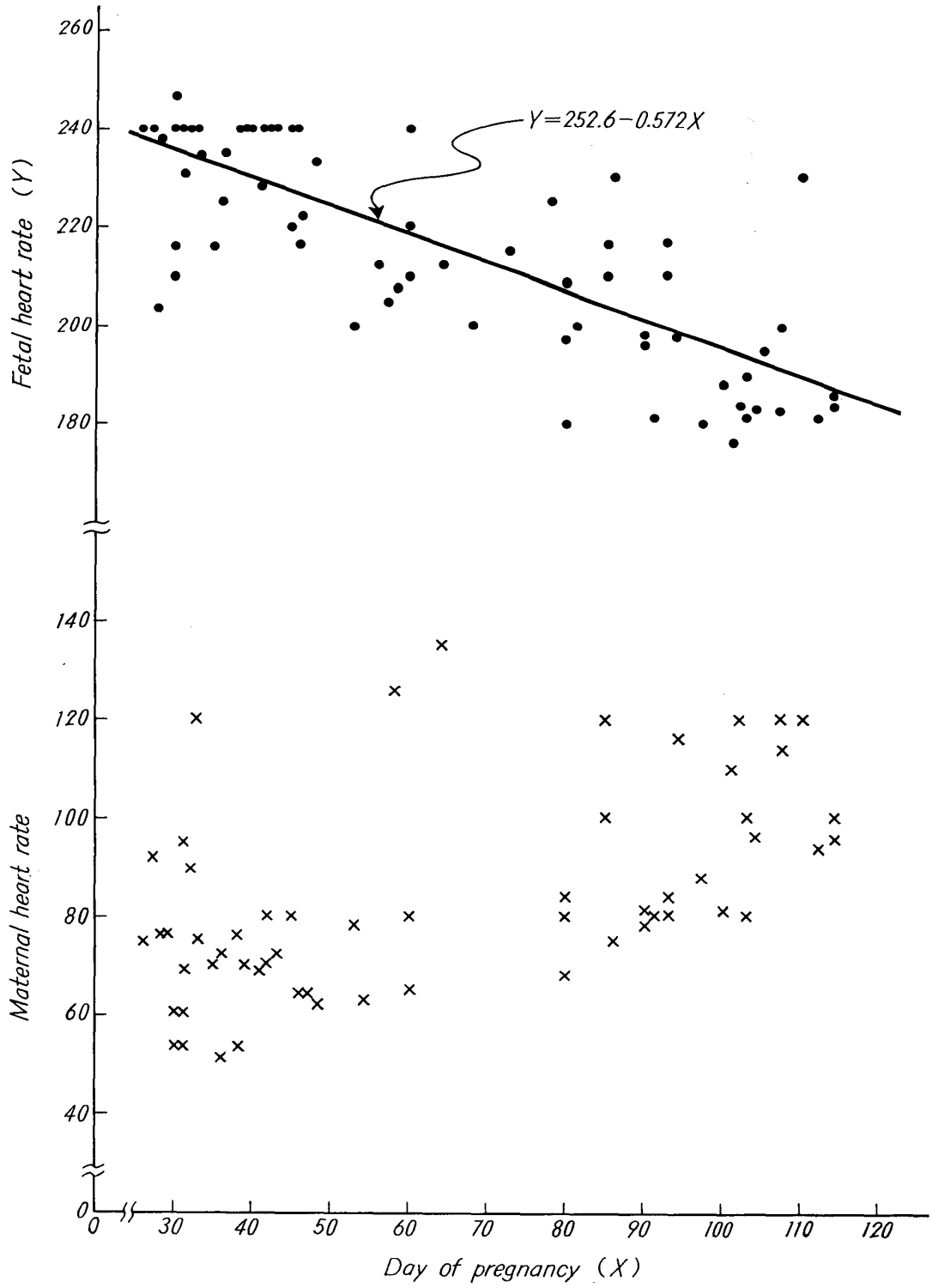


TABLE 2 *Fetal and maternal heart rates in various gestational stages*

DAYS IN GESTATION	FETAL HEART RATE	MATERNAL HEART RATE
26～ 39	232±12*(19)	73±17*(19)
40～ 59	229±10 (14)	75±17 (11)
60～ 79	217±12 (7)	93±30 (3)
80～ 99	203±15 (14)	87±15 (13)
100～114	189±13 (13)	103±14 (12)

* Mean±standard deviation/minute
Figures in parenthese indicate number of cases examined

FIGURE 4 *Correlation between days of pregnancy and fetal or maternal heart rate*



on delivery. In no case was a positive diagnosis of pregnancy found to be incorrect. To the contrary, all cases incorrectly diagnosed were those in which the Doppler signal was negative, in spite of being pregnant. These mis-diagnoses occurred in 9 animals within 39 days after breeding. The earliest detection of pregnancy by this method was in a case of 26 days after copulation. Before 30 days post-coitum, the accuracy rate was not satisfactory. However, it became considerably higher in the period from 30 to 39 days. After 40 days of pregnancy, the accuracy rate of diagnosis reached 100 percent. The maternal circulation was easily distinguishable from the fetal by a markedly slower rhythm (fig. 3).

In the early stages of pregnancy, the flank area, the immediately anterior portion of the patella, was the most suitable place for detecting fetal Doppler signals (fig. 3). After the first third of the gestational period, the fetal life was detectable on wide areas of the ventro-lateral abdomen.

2 Fetal and maternal heart rate changes during pregnancy

The fetal heart rate from 26 days of pregnancy to the term was calculated in the 67 fetal heart Doppler signal positive cases presented in table 1. The maternal heart rate was also measured by the ultrasonic Doppler method. The mean values of fetal and maternal rates in each stage of pregnancy are shown in table 2. The former was markedly greater than the latter; especially in the first half of gestational period, the fetal rate being approximately three times of the maternal. As seen in figure 4, it was evident that the fetal heart rate gradually decreased with the progress of pregnancy, while the maternal rate tended to increase slightly only in the late stages of pregnancy. Thus, a very highly significant negative correlation was observed between the fetal heart rate and the fetal age. The correlation coefficient was -0.87 ($P<0.01$). The fetal heart rate became slower in a negative linear regression as gestation progressed; $Y=252.6-0.572\,X$ (X=Fetal age, Y=Fetal heart rate).

Discussion

Fraser & Robertson (1967) were the first to apply the ultrasonic Doppler method on pregnancy checking of swine, gaining a 75 percent accuracy rate in the period from 6 to 12 weeks of fetal age. The same authors[11] reported again a revised accuracy rate (92 percent) from 6 to 13 weeks. But, in these studies they failed to obtain a positive diagnosis prior to 7.5 weeks. Christiansen & Hansen also achieved a 78 percent diagnostic accuracy by a similar Doppler method, obtaining only false-positive results in all cases up to 6 weeks after last estrus. It should be said that about 82 percent accuracy rate in the present

study in the period from 30 to 39 days in gestation is superior to these 3 reports. More recently FRASER et al. (1971) described a 97 percent level of success in pregnancy diagnoses performed on 300 pigs after 1 month of pregnancy, but the distribution of the number of cases in each gestational stage was not indicated. Although it is not easy to ascertain the reasons why a good result was obtained in the present study, some differences in experimental procedures, such as the position of the animal at examination, the region for detecting the fetal life or the criterion for diagnosis, should be considered. In the experiments of FRASER & ROBERTSON, the standing position was used, while in this study the lying position was taken. The lying position seemed profitable in that the animals were quiet in that position. To detect the fetal life within 40 days of pregnancy, the flank region was preferable, but the other investigators mentioned above did not aim at the flank region. As a criterion for pregnancy diagnosis, CHRISTIANSEN & HANSEN used Doppler signals reflected from the uterine vessels as well as signals from the fetal heart. This would be a cause of the false-positive results prior to 6 weeks of pregnancy in their experiments, because, according to experiences of the present authors, the occurrence of signals from the uterine vessels was not particular with the pregnant uterus.

In the present study, the earliest detection of pregnancy by this method was in a case of 26 days post-coitum. This would give evidence that the Doppler method is surprisingly sensitive for detecting the fetal circulation, in view of the fact that a functionally competent circulatory system is laid down before the developing pig fetus has reached a length of 10 mm[33] (approximate 20 days in the fetal age). In addition, one hundred percent accuracy from 40 days of pregnancy onward would show that this method is not only applicable to early pregnancy diagnosis, but also helpful to the early detection of infertile individuals in swine.

The diagnostic accuracy rate in 30 days or so of gestation, however, was not satisfactory. Further improvement would be needed for such a very early diagnosis. For example, as LINDAHL[27] stated that instruments with a frequency of 5 MHz seem to be superior than those with a frequency of 2.25 MHz for the early detection of pregnancy in dairy goats, it will be necessary to check this also in sows. Furthermore, as already suggested by HULET, the development of a probe with a broader ultrasonic beam without reducing efficiency may be capable of either increasing accuracy or saving time in diagnosis.

WILSON & NEWTON, FRASER et al.[9], KEANE, and LINDAHL[29] applied the ultrasonic Doppler method to the estimation of the number of fetuses in the ewe. The present authors also have made some experiments by the Doppler method to estimate the litter size in utero in the sow, and achieved a good accuracy as

compared with the actual litter size at parturition. Although part of the data has already been reported[12,13], the details together with some additional data will be published in the next paper of this series.

The fact that information on the fetal heart rate in various developmental stages during pregnancy in the swine, which had been almost impossible to obtain in intact, can be easily taken by the ultrasonic Doppler method, seems to be a very significant in fetal physiology and pathology. FRASER et al.[9] first reported about changes of the swine fetal heart rate in the period from 50 days onward to the full term. As for the fetal heart rate in the earlier stages of pregnancy, however, no information has been available. In the present study the authors could fill part of this gap. It is quite interesting that the fetal heart rate is very fast in the early stages of gestation, with a gradual decrease as pregnancy advances, and that there is a very highly significant negative correlation between the fetal heart rate and the fetal age. FRASER et al.[9] also attained similar results in ewes and sows, and confirmed that, in cases where coital data are uncertain, if the regression equation is used, the fetal age can be estimated from the fetal heart rate calculated with the Doppler method, thus the likely parturition date can to some extent be predicted (70 percent of sows farrowed within seven days of the predicted date). The estimation of the birth date will be of value in the management of pregnant animals in these species. Also, checking the fetal pulse rate will give some information about the health conditions of the fetus.

In the present authors' experiment, no detrimental effects of ultrasound were observed in either fetuses or dams. A few sows aborted or farrowed mummified fetuses, but the frequency was rather lower than in the cases of untreated controls. Likewise, there were no descriptions suggesting any harmful effects of ultrasound in the papers of the other authors cited here. SHOJI et al., however, reported that mouse embryos on the 9th day of gestation radiated with a frequency of 2.25 MHz and an intensity of 40 mW/cm^2 of ultrasound waves by a Doppler instrument for 5 hours produced some external abnormalities and increased fetal mortality. One fetus with exencephaly observed was never spontaneously found in mice of this strain. They stated that such data seemed to suggest an increase in the frequency of maldevelopment or intrauterine death in mouse embryos by ultrasound radiation. Although in the practical use of Doppler instruments for pregnancy diagnosis such long-period radiation of ultrasound waves may not be administered, it seems preferable that the radiation of ultrasound waves should be made as brief as possible in the earlier stages of gestation, especially in the organogenesis period.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. T. ISHIKAWA, Professor of the Department of Veterinary Obstetrics of this faculty, and Mr. M. ABE, Chief Scientist of Swine Section, Takikawa Animal Husbandry Experiment Station, for their encouragement. Further thanks are offered to Aloka Co. Ltd. for cooperation with instrumentation.

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