STUDY ON THE RELATIONSHIPS OF INTRAVAGINAL ELECTRICAL IMPEDANCE AND PLASMA LEVEL OF PROGESTERONE AND ESTRADIOL-17B IN ESTRUS SYNCHRONIZED SOWS IN PREDICTION OF THE OPTIMAL INSEMINATION TIME IN PIG

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ABSTRACT
An experiment aimed at determining the association of impedance values with plasma level of progesterone and estradiol-17b in estrus synchronized sows for prediction of optimal insemination time was conducted in Large Animal Clinic for Theriogenology and Ambulatory Service of Faculty of Veterinary Medicine, Leipzig University, Germany. Measurements of intravaginal electrical impedance (IEI) were carried out throughout the estrous cycle (synchronization phase and estrus period) using a 2-electrode impedance-measuring instrument (INOTEC FEG GmbH, Germany). Values of impedance measurements were analyzed based on clinical findings including ultrasonography, and endocrine parameters (plasma concentration of progesterone and estradiol-17b). Blood samples were collected parallel with measuring of IEI in order to determine plasma progesterone and estradiol-17b concentrations using radioimmunoassay (RIA). High values of impedance were measured during synchronization phases/diestrus period, and low IEI values coincided with the pre-ovulatory estradiol-17b peak, at the height of standing heat. IEI values began to decline, right after withdrawal of Regumate administration, about 2 to 3 days before the onset of standing heat. Average low values of impedance were registered within 36 to 52 hours at times of standing heat. Relationship among plasma levels of estradiol-17b and progesterone and values of IEI during estrus period was evident: Low IEI values were significantly correlated to maximum plasma levels of estradiol-17b (r = 0.24) (P < 0.05). Plasma progesterone levels also revealed significant association with IEI values (r = 0.62) (P < 0.01). IEI values were also associated with psychosexual behavioral changes and changes in the reproductive tract during estrus period: congestion and edema of the vulva and vagina began at time of declining values of impedance, during the onset of pre-estrus phase. The average duration of ovulation was 4.1 ± 1.23 hours. This study revealed that based on values of IEI and its indirect association with estradiol-17b, the onset of estrus can be better recognized, and thus, the optimal insemination time can be predicted.

Key words: Estrous cycle, estrus detection, impedance, ovarian steroid hormones, ovulation, pig.

INTRODUCTION
One of the major constraints to good reproductive performance in farm animal reproduction is low estrus detection. In pig farms, failure to identify animals in estrus may result in reduced reproductive performances by contributing to prolonged non-productive days in individual animals, or by increasing...
reproductive problems in the herd (Clark et al., 1986; Love et al., 1993).

Regulation of cyclical activity is mainly under the control of the hypothalamic-pituitary-ovarian axis. Reproductive hormones such as GnRH, gonadotropins, ovarian steroids and inhibin as well as prostaglandin F2α are involved in the controlling of the estrous cycle. The changes in concentrations of reproductive hormones in the peripheral circulation are similar to other farm animals. Estrogens, particularly oestradiol-17B, start to rise at the time that the copora lutea begin to regress, reaching a peak about 48 hours before the onset of estrus in the peripheral circulation. The preovulatory LH peak occurs at the beginning of estrus and eight to 15 hours after the estrogen peak (Van de Weil et al., 1981). The changes in progesterone concentrations mimic the physical changes of the corpora lutea.

In female animals after the onset of puberty there are a series of events in the reproductive tract that occur in a definite order over a period of days. These functional cyclic changes in the organs of female reproductive system are specific for each individual phases of the estrous cycle. Numerous authors (Smith et al., 1989; Lehrer et al., 1991; Imwalle et al., 2007) described not only the changes in the vaginal epithelium but also its electrophysiological characteristics resulting from fluctuation in the concentration of ovarian hormones, particularly from elevated level of estrogen. During pre-estrus period, the vaginal and vulvar tissue become progressively edematous and congested due to the effect of estrogen. Consequently, the electrical conductivity of the mucus and epithelium increases. These features persist throughout the heat and gradually subside during the two or three days afterwards. The histological and biochemical changes of the reproductive tract, which are induced by the ovarian endocrine activity, can be monitored by measuring of impedance. This is the ability of a tissue to resist the flow of an externally applied weak electrical current (Lehrer et al., 1991).

The objective of this study was to determine the relationships between intravaginal electrical impedance (IEI) and plasma concentration of progesterone and estradiol-17b in prediction of optimal insemination time in pig. In line with this, an experiment was conducted with specified experimental design. Values of impedance measurements were analyzed based on clinical findings including ultrasonography, endocrine parameters (plasma concentration of progesterone and estradiol-17b).

**MATERIALS AND METHODS**

An experiment aimed at determining the association of impedance values with plasma level of progesterone and estradiol-17b in estrus synchronized sows for the detection of optimal time of insemination was conducted in Large Animal Clinic for Theriogenology and Ambulatory Service of Faculty of Veterinary Medicine, Leipzig University, Germany. The experiment was conducted in twelve synchronized cyclic sows of German land races with average age of 220 days and body weight of 115 kg for comparison of IEI values with plasma level of progesterone and estradiol-17b. Parallel to blood sampling, IEI measurements were conducted using intravaginal impedance-measuring instrument (INOTEC FEG GmbH, Germany) after cleaning the external genitalia with antiseptics; twice per day at twelve hours interval during the synchronization phase (RegumateÒ administration) and at time of standing heat. Moreover, transabdominal ultrasonic examination (Picker CS 9100-Oculus, 5MHz) was carried out aimed at monitoring of growth of follicles and ovulation process. This was done twice per day during estrus synchronization phase and at four to six hours interval from withdrawal of RegumateÒ administration until the end of ovulation.
Clinical gynecological examinations were performed daily during the pre-estrus period and standing heat: observation of signs of estrus coupled with frequent checking for standing heat by firmly pressing the loin of the sows with palms of both hands.

In order to synchronize the estrous cycle of the sows 0.02g Altrenogest/Regumate was administered orally over 16 days. Twenty-four hours after the last Altrenogest administration, 1000 I.U. PMSG (Pergamonâ) was injected for induction of estrus. This was followed by intra muscular injection of 10 mg of GnRH analogs Buserelin (Receptalâ) to induce ovulation (Möller-Holtkamp et al., 1995). After 24 hours of administration of Receptalâ AI1 and 16 hours after the first insemination AI2 was performed.

In addition, transabdominal ultrasonic examination of the ovaries was carried out once per day during Regumate administration and at four hours interval starting from GnRH administration until the completion of AI1 and AI2 (Fig. 1)

Collection of blood samples and hormone analysis

The collection of blood samples was facilitated through indwelling the external jugular vein using silicon catheter (Dow corning Corp, Midland). Samples were collected once per day (between 07.00 and 08.00 A.M) for determining of progesterone and estradiol-17b concentration using RIA. The blood samples were collected in heprinized test tube and subsequently cooled at room temperature within 10 minutes. After keeping the samples for 15 minutes in refrigerator they were centrifuged at 2000 rpm for 10 min. Then, plasma was pipetted in labeled test tubes and kept at -20 °c until the analysis.

Radioimmunoassay based on H³ was used to analyze the plasma levels of progesterone and estradiol-17b according to Van de Wiel et al., (1981). The analysis was based on the following assay criteria: The sensitivity lies by 11 pmol/l; the inter- and intra-assay variation coefficient amounted 15% and 14.2%, respectively for estradiol-17b, while the sensitivity amounted 0.5 nmol/l; the inter- and intra-assay variation coefficient were 18.5% and 12.3%, respectively for progesterone.

Statistical analysis

In accordance with the explorative data analysis, the data was proved for its normal distribution with the help of Kolmogorov-Smirnov-Statistic. In order to characterize the distribution of values of the parameters (impedance, plasma.
concentration of progesterone and estradiol-17b) the statistical values mean, and standard deviation were calculated. Correlation between IEI values and steroid hormones was also determined. All computations were performed using the computer software package SPSS version 13.0 for Windows. P-values below 0.05 were considered as significant and those below 0.001 as highly significant.

Results and discussion

In this experiment a strong association was observed among the cyclic endocrine changes, ultrasonic ovarian follicular findings and IEI of the vaginal mucus/epithelium in all sows. The curve profile of estradiol-17b and progesterone took the expected course during the whole experiment: Plasma level of progesterone were less than 2 nmol/l in all animals of the group at the end of the synchronization phase, which was followed by rapid increase in the concentration estradiol-17b (100 ± 14 pmol/l). Figure 2 shows plasma concentration profile (X ± s) of estradiol-17b (pmol/l) and progesterone (nmol/l). It was evident that there is temporal relationship between concentration of estradiol-17b and progesterone and IEI values. Positive correlation (r=0.62) (P< 0.01) was registered between IEI values and plasma concentrations of progesterone, whereas negative association (r = -3) (P = 0.05) was observed between the curve profile of the IEI and plasma levels of estradiol-17b (Fig. 2 and 3).

Low IEI values were significantly related to maximum plasma levels of estradiol-17ß (P < 0.05). Plasma progesterone levels have also exhibited an association with IEI values (P < 0.01). As evidenced in fig. 3 high IEI values ranging from 14.86 and 21.97 rel. units were registered during the administration of Regumate®. IEI values began to decline at the end of the synchronization phase, as soon as plasma levels of estradiol-17ß resumed rising. There were significant differences in the values of IEI during the synchronization phase and at time of standing heat: low mean IEI value (1.86 rel. units) was registered

Fig. 2 : Plasma concentrations (X ± s) of progesterone (nmol/l) and estradiol-17ß (pmol/l).
on day three after withdrawal of the RegumateÒ administration, which coincided with the time of AI₁ and AI₂. There was an increase in the values of IEI shortly after ovulation in all animals of the group. At the time when IEI values resumed rising, ovulation process was monitored by means of ultrasonography, which also enabled the determination of duration of ovulation.

It was also possible to monitor the growth and development follicles by means of transabdominal ultrasonic examination technique during the entire phases of the estrous cycle. Initially, smaller follicles ranging from 2 to 6.5 mm in size were observed on both ovaries. Larger follicles were detected two to three days before ovulation so that numbers of follicles with diameter ranging from 6 to 10.5 mm were detected after RegumateÒ withdrawal and during estrus period. The average diameter of the pre-ovulatory follicle was 8.3 mm.

The process of ovulation could also be monitored using transabdominal ultrasonic imaging of the ovaries. Ovulation occurred between 24 and 36 hours after the administration of GnRH. In ten sows, that responded to the treatment ovulation occurred between days 21 and 22 of the estrous cycle, whereas delayed ovulation was registered in two cases where it occurred on day 25 of the cycle. The average duration of ovulation was 4.1 ± 1.23 hours.

Cyclic changes in the mucus membrane of reproductive tract occur as a result variations in the concentration of ovarian hormones, particularly due to increased level of estrogen (Imwalle et al., 2007). Elevated level of estrogen during the follicular phase of the ovarian cycle induces hydration of vaginal and vulvar tissue, which intern increases the electrical conductivity of the vaginal mucus and epithelium (Ezov et al., 1990; Schindler et al., 1990). As evidenced in the results of the present study, there are significant differences in the values of IEI during estrus and synchronization phase/diestrus period. Estrus period is characterized by low values of IEI, whereas high values are registered during the synchronization/diestrus phase of the estrous cycle. Values of IEI tend to increase right after ovulation. Pfandler (1972) and Lehrer et al. (1991) obtained low IEI values apart from estrus period at times of approaching parturition in pregnant sows and cattle.

![Fig. 3](image-url)
respectively. The declining IEI values during parturition in the works of these authors may be induced by increased plasma level of estrogen similar to estrus period.

In the present study, associations among endogenous endocrine changes and internal and external signs of estrus were evident. High blood level of estrogen, in the relative absence of progesterone, initiates the development of internal and external manifestations of estrus as well as induces a positive feedback on the hypothalamus-hypophysis system (Cox and Britt, 1986). According to Walton et al. (1987) external manifestations of estrus coincided with high plasma values of estradiol-17b and LH during the follicular phase of the ovarian cycle. Increased plasma level of these hormones during the pre-ovulatory period and their indirect association to the values of IEI hereby justifies the role of estrogen in the release of LH from the anterior pituitary in the sense of positive feedback. In the current study, high plasma concentration of estradiol-17b as well as low progesterone levels were obtained parallel to low values of IEI. The study reveals a positive correlation between IEI values and plasma concentrations of progesterone on one side and a negative association between estradiol-17β and impedance values on the other side in each sows of the group. These findings are in agreement with results reported by Dusza et al. (1996) and Lewis et al. (1989) who obtained similar findings in sows and cattle, respectively.

In conclusion, the current study disclosed that temporal association among the onset of estrus, estrus related changes in the IEI values, pre-ovulatory estradiol-17β peak and ovulation in pig can be employed to objectively determine the pre-ovulatory period. Based on the values of IEI, the onset of estrus can be better recognized and thus, the optimal insemination time can be predicted.

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