

Oestrone Sulphate Measurements for the Prediction of Small or Large Litters in Pigs

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Gaustad-Aas AH, Ropstad E, Karlberg K, Hofmo PO, Dahl E: Oestrone sulphate measurements for the prediction of small or large litters in pigs. Acta vet. scand. 2002, 43, 157-164. – Serum from 88 pregnant sows and gilts was sampled 24 and 28 days after their first insemination or mating day. The oestrone sulphate (E1S) concentration in the samples was assessed with a commercially available radioimmunoassay kit modified for use with swine serum. The first aim was to test whether it was possible to predict litters of total number <10 piglets at term. The second aim was to compare the use of day 24 or day 28 samples, or of both, in this prediction.

Day 24 E1S levels were positively correlated with litter size at term ($R^2 = 0.26$; $p < 0.001$). E1S levels on day 28 were correlated with the levels on day 24 in the same animals but could not be used for prediction of large or small litters. The odds ratio for a small litter size was 0.16 ($p < 0.01$). This means that odds for a litter size <10 piglets decreased by 84% when E1S levels increased by 1.0 ng/ml.

sows; pregnancy; prediction; litter size; oestrone sulphate.

Introduction

In the pregnant sow, the embryonic units produce oestrone (Lunaas *et al.* 1973, Perry *et al.* 1973, 1976, Gadsby *et al.* 1980) which is conjugated with sulphate groups within the endometrium (Dwyer & Robertson 1980). Its conjugates have been demonstrated from day 17 of pregnancy with increasing concentration until day 28-30 and a subsequent decrease later in pregnancy (Robertson *et al.* 1978, 1985, Chew *et al.* 1979). The concentration of oestrone and its conjugates in the post-breeding female's plasma, serum, urine, faeces and saliva has been assessed in order to diagnose pregnancy (Robertson *et al.* 1978, Hattersley *et al.* 1980, Saba & Hattersley 1981, Atkinson & Williamson 1987, Choi *et al.* 1987, Vos *et al.* 1999, Ohtaki *et al.* 1997). In addition, a correlation between the number of embryos in early pregnancy and the maternal concentration of

oestrone conjugates has been demonstrated (Chew *et al.* 1979, Cunningham 1982, Horne *et al.* 1983, Atkinson *et al.* 1986, Stone *et al.* 1986, Atkinson & Williamson 1987).

A large number of herds in Norway practice a 3 or 7 week batch farrowing system. To compensate for sows with delayed oestrus after weaning and non-pregnant sows, a surplus of gilts and sows are mated to yield the planned number of litters at term. Therefore, it would be beneficial to be able to select pregnant animals with the highest presumptive litter size early in gestation, so that sows and gilts with a presumptive low litter size could be culled. In multiplier herds, prediction of a minimum litter size might increase the value of the animals intended for sale. However, to our knowledge, few studies have evaluated the correlation between maternal oestrone sulphate level and lit-

ter size at term (Stone *et al.* 1986, Stoner *et al.* 1986, Frank *et al.* 1987, Moenter *et al.* 1992). Furthermore, the value of analysing a single blood sample for prediction of litter size at farrowing has been debated (Hattersley *et al.* 1980, Atkinson *et al.* 1986), whereas specifying the interval from oestrus to sampling might improve the results (Stone *et al.* 1986).

Variations in previous lactation length and weaning to service interval may be associated with variation in litter size (Dewey *et al.* 1994, Koketsu & Dial 1997, 1998, Marois *et al.* 2000). Litter size is also highly correlated with parity, and prediction equations for litter size in parity 2 and 3, based on previous litter size, have been derived (Lundeheim & Eliasson-Selling 1996).

The aims of this study were (1) to assess the possibility of predicting small or large litters at term by means of analysing the oestrone sulphate level in blood samples, and (2) to evaluate whether sampling on day 24 and day 28 after the first day of service may improve prediction of litter size, compared to sampling only on day 24.

Materials and methods

Animals

Two trials were performed. In a preliminary trial (trial 1), 5 adult sows were bled at 2 day intervals from day 18 to day 30 post AI.

In a subsequent trial (trial 2), a total of 78 Landrace × Yorkshire sows and 12 gilts from a breeding and pregnancy unit of a sow-pool were included in the study. The animals were bled on days 24 and 28 after mating or AI if return to oestrus was not observed prior to this. Two Landrace × Duroc boars were used for natural mating. Semen used for AI was commercially available^a pooled fresh semen from Landrace × Duroc boars, used on the day of collection or the day after. All the sows and gilts had been heat tested with a boar also if they

were inseminated artificially. Insemination was also performed adjacent to a boar. All the sows included had a weaning to service interval of 4 or 5 days. Parity ranged from 1 to 5.

Parity and the number of AI or matings were recorded and included in the statistical analyses. Subsequent litter sizes, as well as returns to oestrus after blood sampling, were recorded. Only sows and gilts that farrowed were included in the statistical analysis of litter size (n=88).

In both trials, blood was sampled from a prominent ear vein and allowed to clot naturally. The serum was transferred to plastic tubes after centrifugation, and stored at -20 °C until analysis.

Oestrone sulphate assay

The serum was analysed for oestrone sulphate (E1S) by a commercial radioimmunoassay kit "Estrone-sulfate DSL-5400"^{®b} modified for use with swine serum. Modification was done as follows: The standard curve was replaced by E1S diluted in pooled serum from castrated male pigs of approximately 30 kg live weight (0-serum). Dilutions of serum samples with varying concentrations of E1S were parallel with the standard curve.

Inter-assay coefficients of variation in samples containing 3.27, 7.86 and 22.8 ng/ml were 10.9%, 8.9% and 3.8%, respectively. Minimum detection limit in the assay was 0.01 ng/ml.

Statistical analyses

All statistical analysis were performed in SAS (SAS Institute Inc. 1990). Differences in the number of matings or AI between parities were tested with Fisher's exact test using PROC

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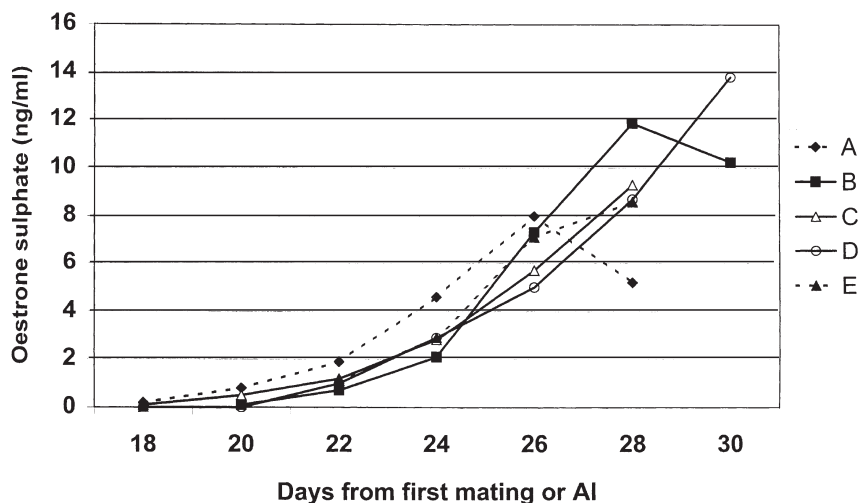


Figure 1. Oestrone sulphate in serum of 5 individual sows bled on alternate days during early pregnancy.

FREQ. Differences in litter size between animals with different numbers of matings or inseminations were tested with the median test using PROC NPAR1WAY. Correlation between day 24 level and day 28 level of E1S was tested using the CORR procedure. Variation in litter size was analysed with the UNIVARIATE procedure.

Analysis of variance was performed with the GLM procedure. Multivariate models were run with the values of E1S on days 24 and 28, respectively, as response variables. In these models the explanatory variables were the fixed effect of parity and regression on actual litter size (total number born).

Litter size was classified in 3 classes, 'class A' (range 3-9 piglets; $n=8$), 'class B' (range 10-14 piglets; $n=48$) and 'class C' (range 15-22 piglets; $n=32$). These classes were used in a GLM model together with parity in order to obtain least squares mean differences between E1S levels on day 24 and day 28.

Logistic regression by PROC LOGISTIC was

used to estimate the probability of a litter size in class A (<10 piglets). E1S level on day 24 and day 28, parity and the number of matings or inseminations were possible explanatory variables in a stepwise selection procedure.

Results

In the preliminary trial, the E1S concentration of the 5 pregnant sows was found to increase markedly from day 22-24 to day 26-28 (Fig. 1).

In trial 2, 12 of the animals were mated or inseminated once, 55 were mated or inseminated on 2 consecutive days, while 23 were mated or inseminated on 3 consecutive days. Mating and insemination work started 4 h earlier for each consecutive day, so that the interval between matings or inseminations was approximately 20 to 22 h.

A total of 77 sows and 11 gilts farrowed with a mean total litter size of 13.8 piglets (s.e.m. = 0.3) and 10.3 piglets (s.e.m. = 0.9), respectively. The difference in subsequent litter size between

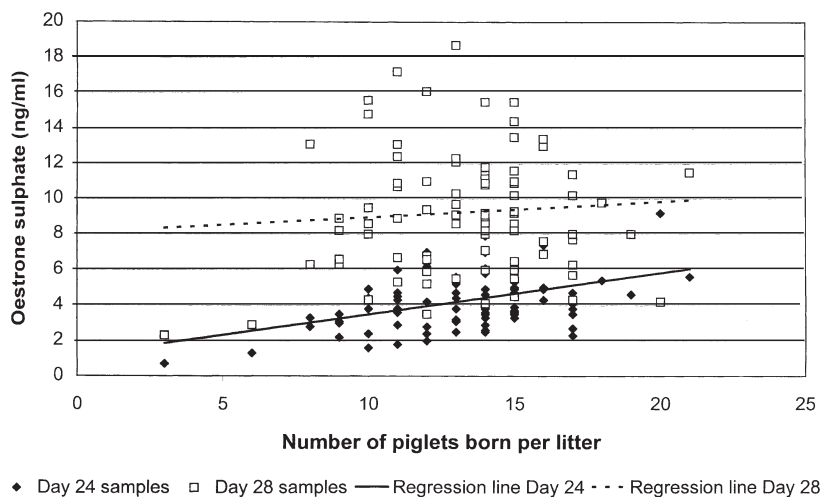


Figure 2. Relationship (raw data) between oestrone sulphate concentration in serum 24 and 28 days after first mating or AI and total number of piglets subsequently born per litter.

gilts and sows was significant ($p < 0.001$). Litter sizes ranged between 3 and 21 piglets. Mean previous lactation length was 33.9 days (s.e.m. = 0.2 days).

The mean number of AI or matings was similar for both gilts and sows (1.82 and 2.17, respectively, $p > 0.10$). Between animal groups with different numbers of AI or matings, the litter sizes were similar (total number born = 13.1, 13.4 and 13.6, for 1, 2 and 3 matings, respectively, $p > 0.10$). No significant differences were found in E1S level on day 24 or on day 28 between animals with different numbers of matings or AI. For triple inseminated or mated animals there was a tendency toward lower E1S levels on day 24 with a proportionately higher increase until day 28 compared to animals inseminated or mated only once ($p = 0.07$ and $p = 0.08$ for day 24 level and percentage increase, respectively).

Mean serum E1S level on day 24 for the 88 pregnant animals was 4.1 ng/ml (s.e.m. = 0.2 ng/ml) while mean level on day 28 was 8.8

ng/ml (s.e.m. = 0.3 ng/ml). The E1S levels on day 24 and day 28 within animal were correlated ($r = 0.35$, $p < 0.001$).

Subsequent litter size was found to have a strong positive linear relationship with the day 24 E1S level ($p < 0.001$), while parity was only slightly correlated ($p < 0.10$). The R^2 of this model was 0.26. Neither litter size nor parity was related to serum levels on day 28 ($p > 0.10$). The relationship between litter size and serum levels of oestrone sulphate on day 24 and 28 is shown in fig. 2.

When litter size was ranged in classes A-C and adjusted for parity, there was a significant relation with day 24 E1S concentration ($p < 0.01$), whereas parity was less strongly related ($p < 0.10$, the R^2 of the model being 0.25). Least squares mean differences between day 24 E1S concentrations in the 3 litter size classes are shown in Table 1. Repeating the model with day 28 E1S concentrations resulted in non-significant parity differences ($p > 0.10$), while litter size classes were significant ($p < 0.05$). How-

Table 1. Least squares mean differences in serum levels of oestrone sulphate between litter size classes A, B and C in sows and gilts 24 days after first AI or mating. (Litter size class A included 3-9 piglets; class B included 10-14 piglets and class C included 15-22 piglets; all numbers representing total number of piglets born.) The model included the fixed effect of parity 1-5 ($p < 0.10$).

Litter size	Differences between least squares means in ng E1S/ml serum (p values)
A vs. B	1.81 ($p < 0.01$)
A vs. C	2.21 ($p < 0.01$)
B vs. C	0.4 ($p > 0.10$)

ever, the only significant difference in this model was between class A and class B.

In the logistic procedure, E1S concentration on day 24 was negatively related and the number of

matings or inseminations tended to be negatively related to the probability of a litter size < 10 piglets (odds ratios for small litters = 0.16 and 0.21; $p < 0.01$ and $p = 0.055$, respectively). The oestrone sulphate level was divided into 5 groups with the mean value and the mean value ± 1 and 2 standard deviations, respectively, as midpoints for each of the groups. Estimated probability curves and proportions of small litters in the proposed E1S classes are shown in Fig. 3.

Discussion

The present study demonstrates that it is possible to differentiate between small litters (< 10 piglets) and large litters (10 or more piglets) on the basis of serum E1S levels on day 24 after the first mating. The study failed, however, to show improved results in the prediction of litter size by including serum concentrations on day 28 in addition to day 24 samples, or by using only day 28 samples. As the hormone is only

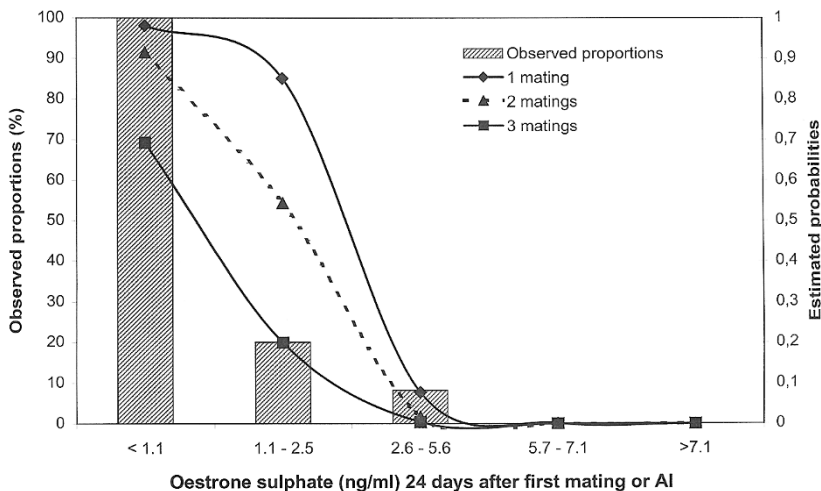


Figure 3. Observed proportions and estimated probabilities of litter size smaller than 10 piglets at term based on serum oestrone sulphate level 24 days after first mating. The probability curves reflect different numbers of consecutive days of mating or insemination.

produced by functional feto-placental units, it was expected that it would be more accurate to assess its serum concentration as late as possible in order to reflect embryo mortality. Embryo losses on day 24, but not on day 30, are reflected in decreased subsequent EIS levels (Horne et al. 1983). Frank et al. (1987) showed correlation between litter size at birth and EIS level on day 28 but not on day 24 within the same animals. However, it has been shown that day 24 levels of EIS have given acceptable correlation with litter size (Horne & Dziuk 1979, Horne et al. 1983, Stone et al. 1986).

The correlation between day 28 and day 24 samples in this study was 0.35, explaining in part why EIS levels on day 28 gave little extra explanation of the variation in litter size. In the preliminary trial some of the animals had decreasing EIS concentration before day 28 while others still had increasing concentrations. This indicated that the peak of the EIS curve may occur before day 28 after first mating or insemination in some cases. Such differences may be due to variations in oestrus duration and interval from the onset of oestrus to ovulation. Another explanation may be embryo mortality in the period between 24 and 28 days. These factors may also partially explain the relatively low correlation between values on day 24 and day 28 in the sow-pool.

At low EIS levels there tended to be a difference in the estimated probabilities of small litters, dependent on how many consecutive days the sow or gilts had been inseminated or mated. A variation in the number of services might be due to variable duration of oestrus, or to variations in oestrous symptoms. Long oestrous periods are correlated to longer intervals from the onset of oestrus to ovulation (Soede et al. 1995, Soede & Kemp 1997, Steverink et al. 1997). This might in its turn mean that some of the triple mated animals had been sampled 2 days later in relation to fertilisation than single

mated animals. The correlation between the number of embryos and EIS level may subsequently have varied, due to the developmental stage of the embryos rather than the number of embryos (Horne et al. 1983). A practical consequence might be to sample animals on a specified number of days from the last insemination instead of from the first.

A relatively small proportion of the litters in the present study was smaller than 10 piglets. Our intention was to evaluate the method in a population of sows and gilts in a field situation, without efforts to alter the variation of litter sizes by surgical or other methods. In some other studies, such efforts have been made, or non-pregnant and/or pseudopregnant animals have been included in the analysis (Horne & Dziuk 1979, Horne et al. 1983, Stone et al. 1986, Stoner et al. 1986). In a field situation, an extra benefit of the proposed method would be the ability to detect non-pregnant animals. This detection is vital in breeding herd management.

Conclusion

The results of the study show that differentiation of small from large litters is possible by analysis of oestrone sulphate levels in the serum of gilts and sows on day 24 post service. Repeated sampling on day 28 does not improve the prediction of litter size. To improve the predictive value for estimation of litter size based on EIS levels, oestrus duration should be taken into consideration. Alternatively, animals with long oestrus duration should be sampled later in relation to the onset of oestrus.

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References

- Atkinson S, Buddle JR, Williamson P, Hawkins C D, Wilson RH: A comparison between plasma oestrone sulphate concentration and doppler ultrasound as methods for pregnancy diagnosis in sows. *Theriogenology* 26(4), 483-490. 1986.
- Atkinson S, Williamson P: Measurement of urinary and plasma estrone sulfate concentrations from pregnant sows. *Dom Anim Endocrinol* 4, 133-138. 1987.
- Chew BP, Dziuk PJ, Thomford PJ, Kesler DJ: Relationships between blood estrone sulfate and fetal number in gilts between days 22 and 80 of pregnancy. *Proc Am Soc Anim Sci 71st Ann Mtg - Abstr.* 365, p. 285-285. 1979.
- Choi HS, Kiesenhofer E, Gantner H, Hois J, Bamberg E: Pregnancy diagnosis in sows by estimation of oestrogens in blood, urine or faeces. *Anim Reprod Sci* 15, 209-216. 1987.
- Cunningham NF: Pregnancy diagnosis in sows based on serum oestrone sulphate concentration. *Br. Vet. J.* 138, 543-544. 1982.
- Dewey CE, Martin SW, Friendship RM, Wilson MR: The effects on litter size on previous lactation length and previous weaning-to-conception interval in Ontario swine. *Prev Vet Med* 18, 213-223. 1994.
- Dwyer RJ, Robertson HA: Oestrogen sulphotase and sulphotransferase activities in the endometrium of the sow and ewe during pregnancy. *J Reprod Fertil* 60(1), 187-191. 1980.
- Frank GR, Noble RC, Esch MW, Green C, Bahr JM: Direct estimation of estrone sulfate in serum and urine of pregnant swine as indicators of litter size at birth. *Anim Reprod Sci* 15, 121-129. 1987.
- Gadsby JE, Heap RB, Burton RD: Oestrogen production by blastocyst and early embryonic tissue of various species. *J Reprod Fertil* 60, 409-417. 1980.
- Hattersley JP, Drane HM, Matthews JG, Wrathall AE, Saba N.: Estimation of oestrone sulphate in the serum of pregnant sows. *J Reprod Fertil* 58, 7-12. 1980.
- Horne C, Dziuk PJ: Relationship between level of estrone sulfate and number of fetuses in gilts from day 10 to 32. *Proceedings, Am Soc Anim Sci 71st Ann Mtg - Abstr.* 403, p. 304-305. 1979.
- Horne C, Chew BP, Wiseman, BS, Dziuk PJ: Relationship between the level of estrone sulfate in the plasma and the number of fetuses during pregnancy in the gilt. *Biol Reprod* 29, 56-62. 1983.
- Koketsu Y, Dial GD: Quantitative relationships between reproductive performance in sows and its risk factors. *Pig News and Information* 18(2), 47N-52N. 1997.
- Koketsu Y, Dial GD: Interactions between the associations of parity, lactation length, and weaning-to-conception interval with subsequent litter size in swine herds using early weaning. *Prev Vet Med* 37, 113-120. 1998.
- Lunaas T, Refsdal AO, Schultz RH: Conjugated oestrone in uterine vein blood during early pregnancy in the pig. *Acta Endocrinol (Suppl. 177, 9th Congress)*, 42-42. 1973. København.
- Lundeheim N, Eliasson-Selling L: Is culling due to a small first litter good business? *Proceedings of the 14th IPVS Congress, Bologna, Italy*, p. 568. 1996.
- Marois D, Brisbane JR, Laforest JP: Accounting for lactation length and weaning-to-conception interval in genetic evaluations for litter size in swine. *J Anim Sci* 78, 1796-1810. 2000.
- Moenter SM, Webel SK, Dziuk PJ: Pregnancy detection and litter size classification by estrone sulfate measurements in swine under farm conditions. *Anim Reprod Sci* 27, 161-167. 1992.
- Ohtaki T, Moriyoshi M, Nakada K, Nakao T, Kawata K: Radioimmunoassay of saliva estrone sulfate in pregnant sows. *Vet Med Sci* 59(9), 759-763. 1997.
- Perry JS, Heap RB, Amoroso EC: Steroid hormone production by pig blastocysts. *Nature* 245, 45-47. 1973.
- Perry JS, Heap RB, Gadsby JE: Endocrinology of the blastocyst and its role in the establishment of pregnancy. *J Reprod Fertil* 25(Suppl), 85-104. 1976.
- Robertson HA, King GJ, Dyck GW: The appearance of oestrone sulphate in the peripheral plasma of the pig early in pregnancy. *J Reprod Fertil* 52, 337-338. 1978.
- Robertson HA, Dwyer RJ, King GJ: Oestrogens in fetal and maternal fluids throughout pregnancy in the pig and comparisons with the ewe and cow. *J Endocrinol* 106, 355-360. 1985.
- Saba N, Hattersley JP: Direct estimation of oestrone sulphate in sow serum for a rapid pregnancy diagnosis test. *J Reprod Fertil* 62, 87-92. 1981.
- SAS Institute: SAS Procedures Guide; Version 6, Third Edition. SAS Institute Inc., Cary, NC. 1990.
- Soede NM, Wetzels CCH, Zondag W, de Koning MAI, Kemp B: Effects of time of insemination relative

- to ovulation, as determined by ultrasonography, on fertilization rate and accessory sperm count in sows. *J Reprod Fertil* 104, 99-106. 1995.
- Soede NM, Kemp B: Expression of oestrus and timing of ovulation in pigs. *J Reprod Fertil Suppl.* 52, 91-103. 1997.
- Steeverink DWB, Soede NM, Bouwman EG, Kemp B: Influence of insemination to ovulation interval and sperm cell dosage on fertilisation in sows. *J Reprod Fertil* 111, 165-171. 1997.
- Stone BA, Seamark RF, Godfrey BM, Quinn P, Lloyd B: Oestrone sulphate levels in plasma of sows as a basis for prediction of litter size at term. *Anim Reprod Sci* 11, 51-62. 1986.
- Stoner CS, Bazer FW, Thatcher WW, Wilcox CJ, Combs GE, Knight JW, Wettemann RP, White CE: Relationship between estrone sulfate in plasma and litter size at farrowing for sows and gilts. *Theriogenology* 25, 709-720. 1986.
- Vos EA, van Oord R, Taverne MAM, Kruip TAM: Pregnancy diagnosis in sows: direct ELISA for estrone in feces and its prospects for an on-farm test, in comparison to ultrasonography. *Theriogenology* 51, 829-840. 1999.

Sammendrag

Målinger av østronsulfat for å forutsi små eller store grisekull.

Serum fra 88 drektige purker og ungpurker ble tatt ut 24 og 28 dager etter første bedekningsdag. Prøvene ble analysert for østronsulfat med et kommersielt tilgjengelig RIA-kit, som var modifisert for bruk på svineserum. Studiens første formål var å teste muligheten for å predikere kullstørrelser på under 10 grisunger totalt, ved fødsel ved fullgått termin. Formål nummer 2 var å sammenligne bruk av prøver fra dag 24 eller dag 28, eller begge, i denne prediksjonen. Nivåene av E1S på dag 24 var positivt korrelert med kullstørrelsen ved fødsel ($R^2 = 0.26$; $p < 0.001$). E1S-nivåene på dag 28 var korrelert med nivåene på dag 24 i samme dyr, men de kunne ikke benyttes til prediksjon av store eller små kull. Odds ratio for et lite kull var 0.16 for E1S (ng/ml serum), ($p < 0.001$). Det vil si at odds for <10 grisunger sank med 84% når E1S-nivåene økte med 1.0 ng/ml.

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