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Changes of Vaginal Epithelium in Creole Pigs Ovulating During Lactation

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Abstract: The objective of this study was to identify changes of the vaginal epithelium in Mexican hairless sows, which ovulated during lactation, caused by the effect of the boar presence and the litter withdrawal. In order to determine the oestrus stage, an exfoliative vaginal cytology and 17β estradiol and progesterone determinations were carried out on the 8 day after the onset of lactation out accompanied with behaviour observations. Four groups of sows were used: Group 1 was not stimulated; Group 2, remained with the boar; Group 3 was separated from its litter for 4 h and Group 4 got both stimuli. Vaginal smear samples were collected every 24 h. for 5 days after stimulus. An ANOVA statistical analysis was performed for repetitive samples during the 5 days of the test. Stimuli used in group 4 caused significant modifications ($P< 0•001$) when compared to Groups 1, 2 y 3. Estradiol levels were higher than 30 pg/ml in Group 4 on day 10 *post partum* and 4.5 ng/ml of progesterone on day 11 and 12 *post partum*. It was evident that 100% of the sows in Groups 1, 2 and 3 did not show oestral activity when relating vaginal cytology with the oestral behaviour and hormone determination of the lactating sows, whereas 100% of the sows in group 4 presented oestrus 72 h. after the stimulus and ovulated 24 to 36 h after the oestrus onset, this was corroborated by estradiol and progesterone determinations, respectively.

Key words: ovulation, lactational oestrus, vaginal cytology, sow, reproduction.

Introduction

Exfoliative vaginal cytology used for the detection of oestrus cycle in pigs is not a common procedure (Becker *et al.*, 1993). Researchers have described several other techniques to detect this physiological stage in pigs (Haynes, 1971).

Measurement of the lactational oestrus of the Mexican Hairless pig in a more comprehensive study has already been reported (Alonso *et al.*, 1998; Alonso *et al.*, 1998; Alonso *et al.*, 1998 and Becker *et al.*, 1993). Vaginal smears of lactating- ovulation induced sows from the breed mentioned above were obtained in order to measure histologic changes in the mucosae to characterize the oestrus stage.

The objective of this study was to identify the effect of the presence of the boar and litter withdrawal on vaginal mucosae in lactating Mexican Hairless sows through the exfoliative vaginal cytology, the onset of oestral behavior and estradiol and progesterone hormone detections.

Materials and Methods

Five boars and four groups of 10 primiparous Mexican Hairless sows were used for this study.

Lactating sows were individually housed in 6 m^2 concrete pens for 28 days, from farrowing to weaning. Daily feed during lactation included 3 kg of a commercial feed (12•5 MJ DE/Kg and 15% of crude protein) *ad libitum*. Once pigs were weaned they were group-housed in a communal pen, and remained together for 18 h; the rest of the time they were taken to an acorn forest. Boars were housed under total confinement in individual 5 m^2 concrete pens.

Stimuli used on day 8 postpartum included boar presence and litter withdrawal following this schedule:

1. Sows were moved to the boar area; 15 min of interaction was allowed, then the sows were returned to their litters (Levis, 1984).
2. Litter withdrawal was done for 4 h consecutive.
3. Using both stimuli, and 3 h after the litter withdrawal, sows were moved to the boar's pen and remained there for 15 min. Thereafter, they returned to their own pens, and their litters were placed back in the corrals 45 min. later.

Experimental handling of the animals is described in Table 1.

Oestrus was detected by observing the behaviour of the sow and by performing exfoliative vaginal cytology (Mota *et al.*, 2000). Signs of oestrus included reddening and swelling of the vulva, restlessness, and the Whitten effect

Table 1: Experimental design

Experimental groups	Treatments	Number of sows
1	Control, without stimuli (C)	10
2	Boar presence (B)	10
3	Litter withdrawal for 4h (LW)	10
4	Boar & litter withdrawn (B+LW)	10

(haunch pressure test) without the boar's presence. This test was done twice a day with an interval of 12 h (morning and afternoon). Once the oestrus behaviour was detected through the haunch pressure test, or with the vaginal epithelium differential count when it exceeded 50% of the superficial cells (Becker *et al.*, 1993), sows were moved to the boar's area so that the physiological stage could be confirmed. Afterwards, natural breeding with a mature boar took place at 12 and 24 h.

Sampling methods:

Vaginal cytology: Vaginal smears were collected every 24 h for 5 days after the stimuli were induced. Samples were obtained with a sterile cotton swab that was introduced via a 16 cm length X 15 mm width sterile plastic cannula. Animal's vulva was previously cleaned. Slides were stained with the Papanicolaou method (Koss, 1979) and evaluated with a microscope at 40x; 200 cells per sample were counted. In order to find the maturation index, vaginal epithelial cells were categorized as parabasal, intermediate and superficial ones. Vaginal cytology was necessary because sows do not always show heat signs, and it was not possible to move the boar into the farrowing pen from groups C and LW, otherwise this would have misled the effects.

Blood Collection and Hormone Assays: Jugular venous samples were collected daily on days 8 to 13 after the onset of the lactation from each sow. The final sample was taken on day 28, in order to evaluate the (progesterone) (P_4) concentration. Oestrogen determination was performed to detect the renewal of the ovarian activity, and P_4 determination to detect the presence of corpora lutea indicating ovulatory oestrus.

Serum P_4 and oestrogens were quantified by a solid-phase RIA commercial kit (Farmacéutica S. A. de C. V. Inc., Lab). The Printz *et al.* (1994) methodology was used for progesterone, and the Howard et al. (1990) one for oestrogen. Within and between-assay coefficients of variation for P_4 and estrogens were 9•1% and 14•3%, and 7•5% and 15%, respectively.

When 17β estradiol concentrations were over 25 pg/mL (Cox and Britt, 1982 and Rowlinson and Bryant, 1982) sows were considered to be in oestrus; P_4 levels over 4•5 ng/ml on day 8 throughout the day 13 were considered to be indicative that ovulation had taken place, and these latter concentrations were also indicative of pregnancy on day 28 (Kirkwood and Thacker, 1998).

Statistical Analysis: To test the effect of the presence of the boar and litter withdrawal on different vaginal cell populations, a variance analysis for repeated measures using the general linear model (GLM) procedure of the statistical analysis system (SAS, 1987) was run. The significance level considered for all statistical tests was $P<0•05$.

Results and Discussion

The vaginal cytology test to detect oestral cycle stages in the sow has not been a common practice due to the fact that oestral cycle phases are not too marked, and that its interpretation requires a lot of experience (Haynes, 1971). Betteridge and Raeside (1962) determined that vaginal cytology was not a practical tool to determine oestrus phase in confined sows, as it required the sampling of vaginal smears every 12 h, and also because in each reading at least a count of 100 cells should be detected. Therefore the use of a boar is a more practical, feasible and quick method in intensive production pig farms in order to detect oestrus. Becker et al. (1993), had acknowledged that this technique is very useful in experimental phases when oestrus needs to be detected. Nonetheless, due to experimental reasons a boar cannot be used, principally because there are sows that ovulate without showing any evident oestrus signs, the detection of cell changes at vaginal level is therefore, a key methodology (Hernandez *et al.*, 1999).

The effect of the boar's presence and the litter withdrawal on enucleated superficial, intermediate and parabasal cells is presented in Figs. 1, 2 and 3, respectively.

Sows of Group 1 (C) did not show changes in the vaginal mucosae during the sampling.

Boar's presence (B) only promoted significant changes ($P< 0•05$) in superficial cells on day 4 post stimulus but these changes did not end on the onset of oestrus, which was corroborated by basal levels of 17β estradiol. Temporary weaning (LW) promoted significant changes ($P< 0•05$) when compared to treatments 1 and 2, in the number of intermediate, superficial and enucleated cells on day 10 postpartum, and on parabasal and superficial cells on day

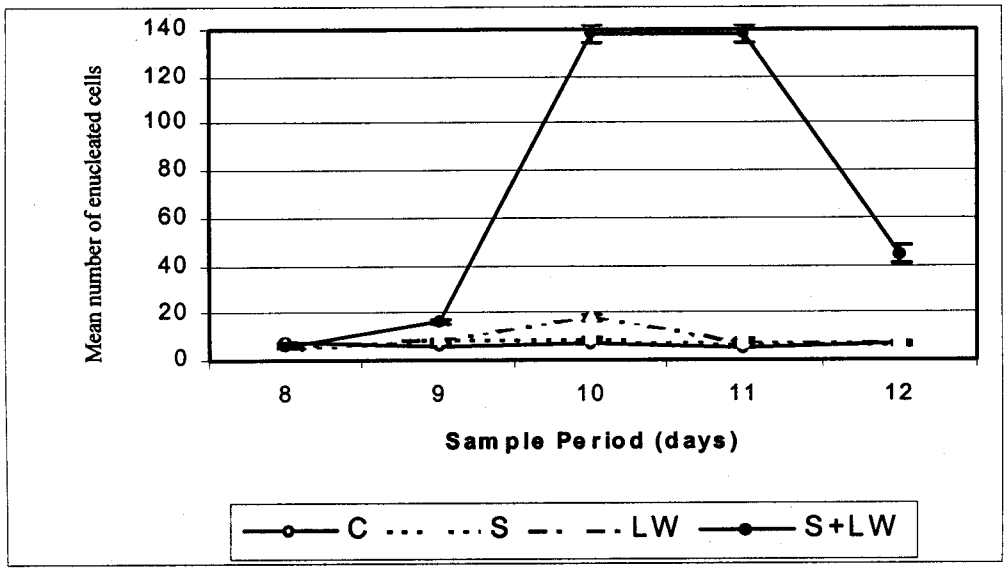

Fig. 1: Effect of treatment on the mean number (± S. E. n = 10 sows per treatment) of enucleated cells. C: Control groups; B: boar stimulus; LW: Litter withdraw stimulus; B + LW: boar and litter withdraw stimuli

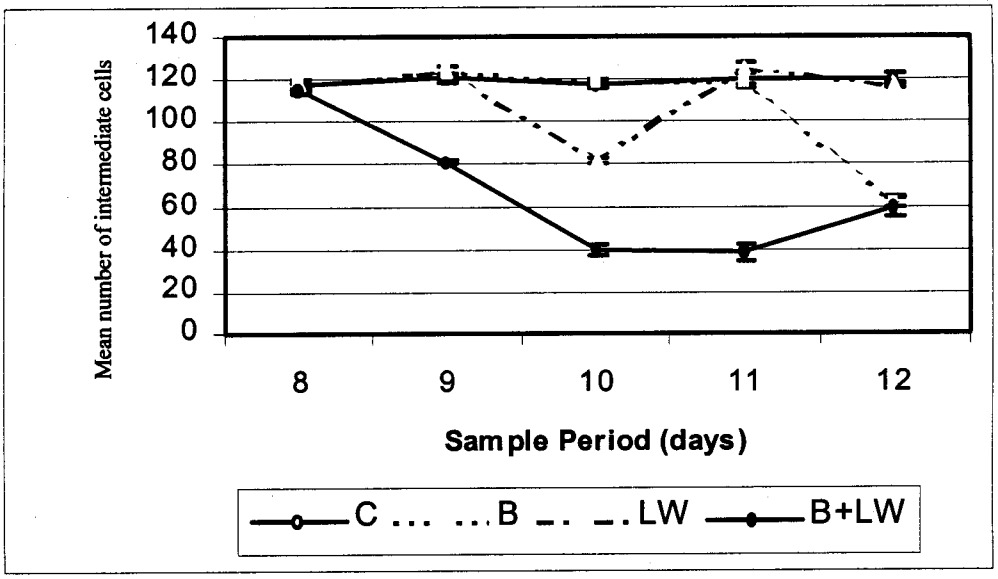

Fig. 2 Effect of treatment on the mean number (± S. E. n = 10 sows per treatment) of intermediate cells. C: Control group; B: boar stimulus; LW: litter withdrawn stimulus; B+LW: boar and litter withdraw stimuli

11, but at the same time these changes were not enough to evidence the reonset of the ovaric activity.
Stimuli used in Group 4 (B+LW), caused significant modifications ($P < 0{\cdot}001$) when compared to groups 1, 2 and 3; nevertheless, the promoted changes in the vaginal mucosae were more marked and permanent than those found in Group 3 (Figs. 1-3). Levels of estradiol superseded the 30 pg/ml on day 10 *post partum*, just one day before the onset of oestrus.
Progesterone's concentration maintained its basal levels, and did not show significant statistical changes in Groups 1, 2 and 3; nevertheless, in Group 4, the plasmatic P_4 concentration superseded the 4·5 ng/ml in the 10 sows within this group 24 to 36 h after the onset of lactational oestrus. In the samples collected on day 28 of the lactation, 8 to 10 sows increased their P_4 levels, and 2 sows reduced these levels below the 4·5 ng/ml; both of them did not reach gestation.

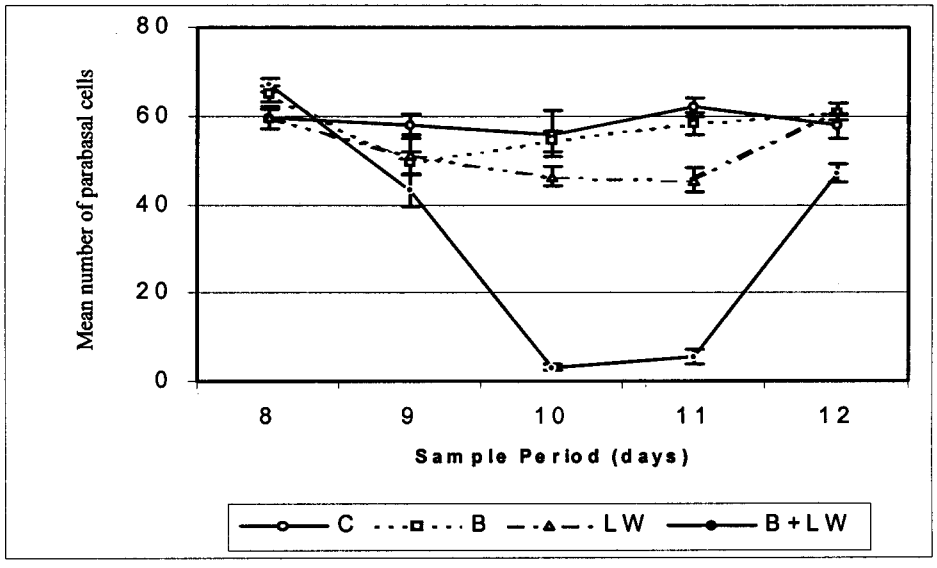

Fig. 3: Effect of treatment on the mean number (± S. E. n = 10 sows per treatment) of parabasal cells. C: Control group; B: boar stimulus; LW: litter withdrawn stimulus; B+LW: boar and litter withdrawn stimuli

It is concluded that the vaginal cytology technique used in this study was useful, as significant cellular changes in the vaginal epithelium of the sows, which reinitiated ovaric activity, were identified, but maybe this technique was not sufficiently reliable to detect the exact moment in which the sow was really on oestrus.

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