Acta vet. scand. 1981, *22*, 67—77.

From the Department of Reproductive Physiology and Pathology,
Veterinary College of Norway, Oslo.

PLASMA PROLACTIN IN THE SOW WITH EMPHASIS ON VARIATION IN RESUMPTION OF OVARIAN ACTIVITY AFTER WEANING*

By
Edvard Benjaminsen

BENJAMINSEN, EDVARD: *Plasma prolactin in the sow with emphasis on variation in resumption of ovarian activity after weaning.* Acta vet. scand. 1981, *22*, 67—77. — Plasma prolactin in the sow was studied during pregnancy, lactation and during the post weaning period. The ovarian activity was monitored by progesterone determinations. In the pregnant sows (2 sows) there was a distinct increase in prolactin values about a week before parturition, amounting to maximum levels around parturition. A moderate decrease in prolactin values during the lactation period (3 sows) was observed. After weaning the prolactin values dropped immediately to basal levels. Of the 91 sows studied in the post weaning period 72 sows resumed cyclic ovarian function within normal time (10 days) after weaning while 19 sows had delayed resumption of ovarian activity. The prolactin patterns in sows with prolonged periods of ovarian inactivity were similar to those seen in normal sows. None of our results indicate that post weaning anoestrus is caused by prolonged hyperprolactinaemia after weaning.

prolactin; sow; pregnancy; parturition; lactation; post weaning anoestrus.

In some mammalian species prolactin seems to exert an inhibitory effect on the ovarian activity (rat, sheep, woman), probably by increasing the sensitivity of the hypothalamus to the negative feedback effects of gonadal steroids (*McNeilly* 1980). In the sow there is usually no cyclic ovarian activity during lactation (*Edquist et al.* 1974). This could be due to the high plasma prolactin levels at this time (*Bevers et al.* 1978, *Landeghem & Wiel* 1978).

* Financial support was given by the Norwegian Agricultural Research Council.

Normally the sow ovulates within 10 days post weaning, but it is not uncommon that the resumption of ovarian activity is delayed for several weeks (*Benjaminsen & Karlberg* 1981). One reason for this could be persisting hyperprolactinaemia after weaning. Such a mechanism would be somewhat similar to the amenorrhoea and infertility associated with pathological hyperprolactinaemia in women (The Chiari Frommel Syndrome) (*Ensor* 1978, *Baird* 1979).

The main purpose of this work was to study if prolonged ovarian inactivity in the post weaning sow is associated with hyperprolactinaemia. Plasma prolactin levels during gestation and lactation were also studied.

MATERIALS AND METHODS

Plasma prolactin levels in the post weaning period were studied in 91 sows. First sample was taken in the last week before weaning and then weekly until assumed pregnancy. All sows were weaned 6 weeks after parturition.

Plasma prolactin levels during pregnancy and lactation were studied in 5 sows. Two sows were blood sampled, usually once a week, during the last 2 months of pregnancy and during the first 3 weeks after farrowing. Farrowing was induced in these sows by 10 mg prostaglandin $F_{2\alpha}$ (Dinolytic® "Upjohn") given intramuscularly on day 113. The other 3 sows were blood sampled twice a week, from a few days after farrowing until approximately 2 weeks after weaning.

For blood sampling the animals were restrained by a snout rope, and V. jugularis was punctured using Vacutainers® (Becton, Dickinson, New Jersey, USA) (*Benjaminsen & Karlberg* 1979).

Plasma prolactin was determined by a RIA as described by *Landeghem & Wiel* (1978). The only difference from the original description was that 50 µl of a porcine plasma was added to the standards 10 min before adding the second antibody in order to minimise the unspecific binding to the second antibody. Incubation with the second antibody was performed at room temperature for 2 h. A 0.05 mol/l phosphate buffer with 0.2 % bovine serum albumin, 0.05 % sodium-azide and pH 7.5 was used in the assay.

The specificity of the antibody has been characterized (*Lan-*

deghem & Wiel). For iodination and preparation of standards, porcine prolactin (pPRL, NIH-SP-162-C) was used.

Initially iodination was performed according to the method described by *Redshaw & Lynch* (1974), later an enzymobead reagent from Bio-Rad was used (*Haug et al.* 1981). The latter method gave higher specific activity and a better immunoreactive material.

Second purification was done each week on a Ultrogel®, ACA 54 column, 1.5 × 30 cm (LKB, Sweden).

The intra-assay coefficient of variation of duplicate determinations was 13.0 %, 5.6 % and 6.1 % in the ranges < 10 ng/ml, 10—40 ng/ml and > 40 ng/ml, respectively ($n = 30$). The inter-assay precision was estimated by duplicate determinations of 3 plasma samples containing different amounts of prolactin. The mean values measured were 4.9 ng/ml, 32.1 ng/ ml and 56.7 ng/ml with a coefficient of variation of 20.6 %, 9.7 % and 9.6 %, respectively ($n = 16$). Sensitivity ($P = 0.01$) was found to be 1.7 ng/ml (sensitivity $= ts/\sqrt{n}$, $t = 2.57$, $s =$ intra-assay precision for samples containing less than 10 ng/ml, $n = 2$) (*Brown et al.* 1957). The recovery of prolactin added to serum amounted to 106 %.

To see if the stress during sampling did affect the plasma prolactin concentrations, 3 sows were continuously restrained for 15 min in the same way as for blood sampling, and samples for plasma prolactin determinations were taken.

Plasma progesterone was measured in all sows in order to monitor the ovarian function. The RIA used has previously been described (*Benjaminsen & Karlberg* 1981).

In the post weaning sows oestrus was recorded by the stockman. Time of observed oestrus was compared with the progesterone profiles. In cases with "silent heat" the approximate time for ovulation was determined according to the progesterone profile.

RESULTS

Plasma prolactin values in sows stressed for 15 min are shown in Table 1.

Fig. 1 shows prolactin concentrations during pregnancy and early lactation in 2 sows. There seemed to be a small increase from about 80 days in the gestation in sow No. 1. In both sows there was a distinct increase in prolactin values about a week

Table 1. Plasma prolactin (PRL) in 3 sows stressed for 15 min.

Duration of stress (min)	PRL (ng/ml)		
	sow A	sow B	sow C
0	12.0	6.4	7.1
2	20.0	6.8	8.4
4	19.8	7.2	9.2
6	16.4	8.2	9.4
10	10.8	7.2	10.0
15	42.0	7.4	9.0

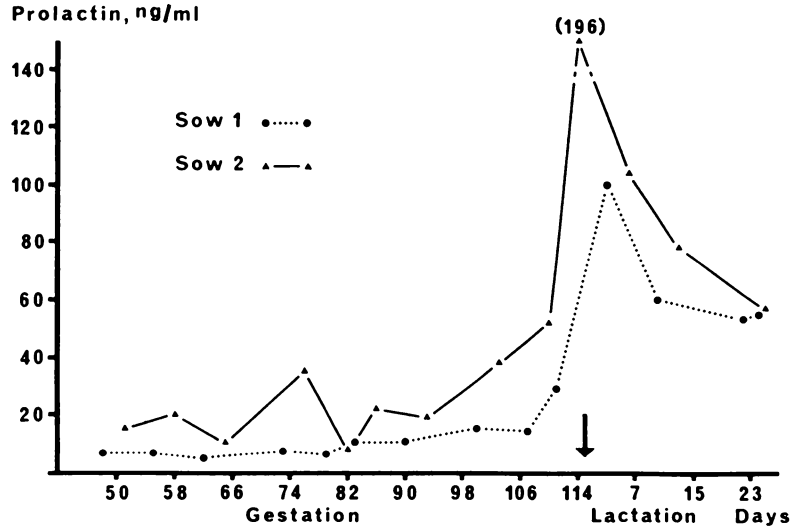

Figure 1. Plasma prolactin in 2 sows during second half of gestation and during the first 3 weeks of lactation.

before parturition and highest values were reached near the time of delivery. The highest value found was in sow No. 2 one day before parturition.

In Fig. 2 prolactin profiles in 3 sows during lactation and after weaning are shown. There was a moderate decrease in concentrations during the lactation period. After weaning there was an abrupt fall in plasma levels. The day after weaning plasma prolactin concentrations were at basal levels.

Of the 91 sows that were observed through the post weaning period, 19 sows had delayed resumption of cyclic ovarian activity as indicated by progesterone levels in plasma. The mean weaning-to-ovulation period in these sows were 30—35 days (range 12—

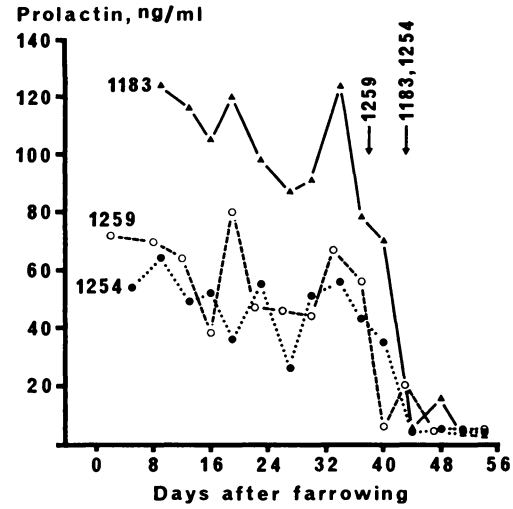

Figure 2. Plasma prolactin in 3 sows during lactation and after weaning.

55 days), while the other 72 sows resumed ovarian activity within 10 days post weaning. Mean plasma prolactin levels before and after weaning in the normal and in the delayed group are illustrated in Fig. 3. There was no significant difference in plasma prolactin levels between the 2 groups.

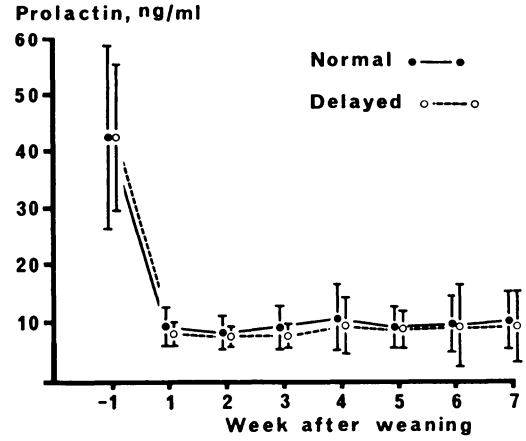

Figure 3. Plasma prolactin (mean ± s) in 72 sows with normal weaning-to-oestrus periods and in 19 sows with delayed resumption of ovarian activity after weaning.

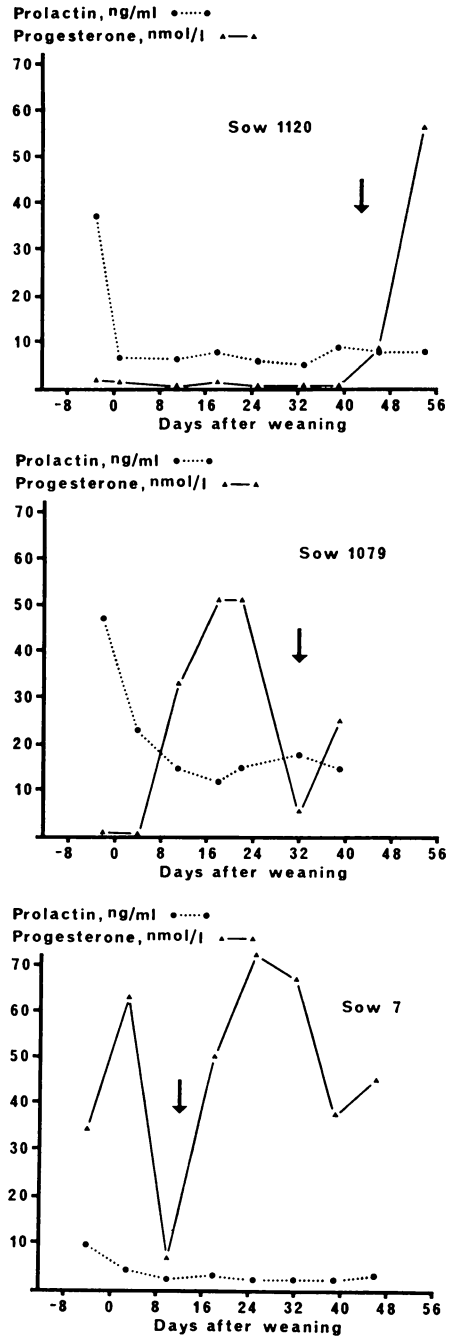

Figure 4. Plasma prolactin in 3 sows with different reproductive patterns. Arrows indicate overt oestrus.

Sow No. 1120: Delayed resumption of ovarian activity after weaning concomitant with low prolactin levels.
Sow No. 1079: Somewhat high prolactin levels but normal weaning-to-ovulation period.
Sow No. 7: Ovulation before weaning associated with low preweaning prolactin value.

The prolactin patterns around weaning in the sows studied were very uniform with no examples of particularly high post weaning levels. Prolactin profiles in 3 individual sows with different reproductive patterns are demonstrated in Fig. 4. In sow No. 1120 plasma prolactin values were low after weaning, but the resumption of ovarian activity was markedly delayed. Sow No. 1070 had the consistently highest prolactin level after weaning. However, the ovarian activity was resumed shortly after weaning. The lowest preweaning prolactin value was found in sow No. 7. This sow was the only one that had ovulated before weaning.

Prolactin levels in the last week before weaning were not found to be correlated with the number of suckling piglets.

DISCUSSION

When collecting blood samples for prolactin determinations one has to consider that stress may induce prolactin secretion (*Ferland et al.* 1978, *Mattheij & Swarts* 1980). One sow (A) showed increased prolactin values after being stressed for 15 min, possibly within 2 min (Table 1). In the other 2 sows there was no change in plasma prolactin concentrations during the period of stress. The results indicate that restraining with a snout rope and puncture of the jugular vein, a procedure usually taking about 1 min, does not to any great extent influence plasma prolactin concentrations. The results suggest, however, that in some individuals stress may induce prolactin release.

The plasma prolactin values obtained during pregnancy and lactation are consistent with values reported by others (*Landeghem & Wiel* 1978, *Smith & Wagner* 1980). The latter authors found increasing prolactin values from 5—6 days pre partum and maximum values 1 day before parturition. *Taverne et al.* (1978/79) recorded distinct increase of prolactin from about 30 h before parturition with maximum concentrations usually a few hours before delivery of the first piglet.

The prolactin levels during lactation obviously can differ considerably between animals (Fig. 2). As suckling is shown to induce prolactin secretion (*Landeghem & Wiel*), the plasma level of prolactin might be influenced by the number of suckling piglets. Sow No. 1254 had considerably lower prolactin level than sow No. 1183, but both had 10 piglets 3 weeks after farrowing.

Neither did the preweaning values in the other sows studied indicate any correlation between number of suckling piglets and prolactin levels during lactation. This is in agreement with the results of *Bevers et al.* (1978).

A rapid fall in plasma prolactin concentrations after weaning seems to occur very constantly. Previously it has been shown that a basal level is reached within 5—6 h after weaning (*Landeghem & Wiel, Bevers et al.*).

It is unclear whether it is the suckling stimulus, the elevated prolactin levels, a combination of these or perhaps other factors which are important for the maintenance of lactational anoestrus in the sow. The hyperprolactinaemia seems to play the major role in the ewe (*Kann et al.* 1977), but not necessarily in the cow (*Peters et al.* 1979, *Gimenez et al.* 1980). In the rat both the suckling stimulus and prolactin seem to be important in inhibiting ovarian cyclicity during lactation (*Smith* 1978). It was interesting to notice that the sow that ovulated before weaning (Fig. 4, sow No. 7) had low preweaning plasma prolactin, indicating a connection between prolactin and the ovarian function in the lactating sow.

There was no difference in plasma prolactin levels between sows with normal ovarian activity after weaning and sows with a long period of ovarian inactivity after weaning (Fig. 3). A typical example of prolactin levels in a sow with delayed resumption of ovarian activity is examplified in sow No. 1120 (Fig. 4). One day after weaning plasma prolactin was at basal level and the values stayed low, but ovarian activity did not return until about 45 days after weaning. In sow No. 1079 (Fig. 4) the decrease in plasma level after weaning was marked, but the decline was protracted and the level stayed somewhat high. The progesterone profile showed that the slightly elevated prolactin concentrations seemed not to have affected ovarian activity. The sow formed corpora lutea within normal time after weaning. Thus, none of our results indicate that hyperprolactinaemia is implicated as a cause of post weaning ovarian inactivity in the sow. This is consistent with a previous report where 4 sows observed in oestrus 16, 20, 27 and 39 days after weaning had plasma prolactin patterns similar to those in sows with normal weaning-to-oestrus intervals (*Wiel et al.* 1979).

In the present study all sows were weaned after 6 weeks of lactation. When the lactation period is reduced the tendency to

prolonged weaning-to-oestrus period is increased (*Svajgr et al.* 1974). It is possible that in sows with a short lactation period hyperprolactinaemia may persist for some time after weaning and thus retard the ovarian activity in some of these sows. However, in a few sows studied, plasma prolactin followed the same pattern irrespective of the length of lactation (*Wiel et al.*).

The reason for prolonged ovarian inactivity in post weaning sows remains unclear. Since, in a few reports, plasma gonadotrophin levels in sows with prolonged weaning-to-oestrus period have been found to be comparable with those seen in normal sows (*Aherne et al.* 1976, *Wiel et al.*), one could suspect a gonadal insensitivity to gonadotrophic stimulation. This is not consistent with the fact that ovulation can be induced with exogenous gonadotrophins in many anoestrous sows (*Hurtgen & Leman* 1979). The possibility that a hypothalamic disorder in the oestrogen feedback mechanism exists in these sows, as discussed in women (*Baird* 1979), remains to be explored.

ACKNOWLEDGEMENTS

The antiserum was produced and provided by Dr. D. F. M. van de Wiel, Research Institute for Animal Husbandry, "Schoonoord", The Netherlands. Porcine prolactin was provided by the National Pituitary Agency, USA, through the kind cooperation of Dr. R. Mäkelä, University of Helsinki. Iodination was performed at the Hormone and Isotope Laboratory, Aker Hospital, Oslo. The animals were made available by the Department of Animal Genetics and Breeding, Agricultural University of Norway.

REFERENCES

Aherne, F. X., R. J. Christopherson, J. R. Thompson & R. T. Hardin: Factors affecting the onset of puberty, post-weaning estrus and blood hormone levels of Lacombe gilts. Canad. J. Anim. Sci. 1976, *56*, 681—692.

Baird, D. T.: Endocrinology of female infertility. Brit. med. Bull. 1979, *35*, 193—198.

Benjaminsen, E. & K. Karlberg: Blodprøvetaking av gris. (Blood sampling in the pig). Norsk Vet.-Tidsskr. 1979, *91*, 15—17.

Benjaminsen, E. & K. Karlberg: Post weaning oestrus and luteal function in primiparous and pluriparous sows. Res. Vet. Sci. 1981, in press.

Bevers, M. M., A. H. Willemse & Th. A. M. Kruip: Plasma prolactin levels in the sow during lactation and the postweaning period as measured by radioimmunoassay. Biol. Reprod. 1978, *19*, 628—634.

Brown, J. B., R. D. Bulbrook & F. C. Greenwood: An evaluation of a chemical method for the estimation of estriol, oestrone and oestradiol-17β in human urine. J. Endocr. 1957, *16*, 41—48.

Edquist, L. E., S. Einarsson & I. Settergren: Ovarian activity and peripheral plasma levels of oestrogens and progesterone in the lactating sow. Theriogenology 1974, *1*, 43—49.

Ensor, D. M.: Prolactin and the primates. *In* Comparative Endocrinology of Prolactin. Chapman and Hall Ltd, London 1978, pp. 198—201.

Ferland, L., G. S. Kledzik, L. Cusan & F. Labrie: Evidence for a role of endorphins in stress-and suckling-induced prolactin release in the rat. Molec. cell Endocr. 1978, *12*, 267—272.

Gimenez, T., D. M. Henricks, A. R. Ellicott, C. H. Chang, J. D. Rone & L. W. Grimes: Prolactin and luteinizing hormone (LH) release throughout the postpartum period in the suckled first-calf beef cow. Theriogenology 1980, *14*, 135—149.

Haug, E., A. Aakvaag, S. Sunde, K. M. Gautvik & K. B. Erik-Nes: Androgen metabolism and mechanism of action in prolactin secreting rat pituitary cells in culture. Submitted to J. Steroid Biochem.

Hurtgen, J. P. & A. D. Leman: Use of PMSG in the prevention of seasonal post-weaning anestrus in sows. Theriogenology 1979, *12*, 207—214.

Kann, G., J. Martinet & A. Schirar: Modifications of gonadotrophin secretion during natural and artificial hyperprolactinaemia in the ewe. *In* P. G. Crosignani & C. Robyn, eds. Prolactin and Human Reproduction. Academic Press, London 1977, pp. 47—59.

Landeghem, A. A. J. van & D. F. M. van de Wiel: Radioimmunoassay for porcine prolactin: Plasma levels during lactation, suckling and weaning and after TRH administration. Acta endocr. (Kbh.) 1978, *88*, 653—667.

Mattheij, J. A. M. & H. J. M. Swarts: Circadian variations of the response of plasma prolactin to brief exposure to ether in the male rat. Acta endocr. (Kbh.) 1980, *94*, 327—331.

McNeilly, A. S.: Prolactin and the control of gonadotrophin secretion in the female. J. Reprod. Fertil. 1980, *58*, 537—549.

Peters, A. R., S. Vyvoda & G. E. Lamming: Comparison of plasma prolactin levels in the milked and suckling cow. J. Endocr. 1979, *83*, 27—28.

Redshaw, M. R. & S. S. Lynch: An improved method for the preparation of iodinated antigens for radioimmunoassay. J. Endocr. 1974, *60*, 527—528.

Smith, B. B. & W. C. Wagner: Lactation physiology of the pig. Proc. 9th Int. Congr. Anim. Reprod., Madrid 1980, vol. III, p. 118.

Smith, M. S.: The relative contribution of suckling and prolactin to the inhibition of gonadotropin secretion during lactation in the rat. Biol. Reprod. 1978, *19*, 77—83.

Svajgr, A. J., V. W. Hays, G. L. Cromvell & R. H. Dutt: Effect of lactation duration on reproductive performance of sows. J. Anim. Sci. 1974, *38,* 100—105.

Taverne, M., A. H. Willemse, S. J. Dieleman & M. Bevers: Plasma prolactin, progesterone and oestradiol-17β concentrations around parturition in the pig. Anim. Reprod. Sci. 1978/79, *1,* 257—263.

Wiel, D. F. M. van de, A. A. J. van Landeghem, A. H. Willemse & M. M. Bevers: Endocrine control of ovarian function after weaning in the domestic sow. J. Endocr. 1979, *80,* 69.

SAMMENDRAG

Plasma prolaktin hos purke spesielt i relasjon til ovarialaktiviteten etter avvenning.

Plasma prolaktin hos purke ble undersøkt under drektighet, laktasjon og i perioden etter avvenning. Purkenes ovarialaktivitet ble undersøkt ved hjelp av progesteronmålinger. Hos de drektige purkene (2 purker) var det en klar stigning i prolaktinnivåene cirka en uke før grising og de høyeste verdiene ble funnet i tiden like rundt partus. I løpet av laktasjonen (3 purker) ble det registrert et moderat fall i prolaktinverdiene. Etter avvenning gikk konsentrasjonene av prolaktin i plasma raskt ned til basalnivået. Av de 91 purkene som ble undersøkt i perioden etter avvenning, ovulerte 72 purker innen 10 dager etter avvenning, mens 19 purker hadde forsinket igangsetting av ovarialfunksjonen. Purkene med forsinket igangsetting av syklisk ovarialfunksjon etter avvenning hadde samme prolaktinmønster som purker som gjenopptok ovarialfunksjonen etter normal tid. Resultatene tyder ikke på at anøstrus hos purke etter avvenning skyldes hyperprolaktinemi.

(Received November 17, 1980).

Reprints may be requested from: Edvard Benjaminsen, the Department of Reproductive Physiology and Pathology, Veterinary College of Norway, P.O. Box 8146 Dep., Oslo 1, Norway.