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Effect of Tetracosactid on Post Partum Cyclicity in Cows after Induction of Parturition with $PGF_{2\alpha}$

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[1]Department of Obstetrics and Gynaecology, Centre for Reproductive Biology in Uppsala, Swedish University of Agricultural Sciences, Uppsala, and [2]Swedish Dairy Association, Eskilstuna, Sweden.

Veronesi MC, Kindahl H, Gustafsson H, Kornmatitsuk B: Effect of tetracosactid on post partum cyclicity in cows after induction of parturition with $PGF_{2\alpha}$. Acta vet. scand. 2001, 42, 243-250. – Parturition and retention of fetal membranes were induced with $PGF_{2\alpha}$ in 3 primiparous dairy cows. Starting on day 12 post partum (PP) the cows were treated with 500 μg i.m. of ACTH-analogue (tetracosactid) every 6 h for 6 times. Changes in plasma concentrations of cortisol, progesterone and 15-ketodihydro-$PGF_{2\alpha}$ were evaluated immediately after treatment. The effects on the resumption of ovarian activity were evaluated by clinical and ultrasound examinations and by progesterone and 15-ketodihydro-$PGF_{2\alpha}$ analyses for 56 days after parturition. Treatment was able to induce a statistically significant ($p<0.01$) similar increase in cortisol and progesterone after both the 1st and the 6th injections, in all cows. No changes in 15-ketodihydro-$PGF_{2\alpha}$ concentrations were seen after any of the injections of ACTH-analogue. The first corpus luteum (CL) was seen on day 18 PP (cow A), and 28 (cow B) and in both cases it was followed by a normal ovarian cyclicity. No CL was observed during the whole period of study in cow C. Progesterone profiles confirmed these clinical and ultrasonographic findings. The steroid output, especially progesterone, induced by the ACTH-analogue might be a stimulus for the onset of ovarian cyclicity, since 2 of the 3 animals ovulated earlier than expected. These findings point to the fact that interference with the stress system might have a positive effect on ovarian cyclicity. The different pattern of response does however demand further studies.

progesterone; cortisol; prostaglandin; metabolite; ACTH.

Introduction

One of the most important indices to evaluate the reproductive efficiency in cattle is the calving interval that could be divided into 2 components: the gestation period and the calving to conception interval. The last one is the parameter usually manipulated in order to achieve the target calving interval (*Peters & Ball* 1995a). The most relevant component of the calving interval is the re-establishment of ovarian cycles after parturition. In normal cycling dairy cows, the length of the interval between calving and the 1st ovulation is variable and affected by many factors such as milk yield, nutrition, body weight and body condition, season and uterine involution. The effects of these factors on the reproductive system are mediated by the endocrine system and probably they can act via a common final mechanism (*Peters & Ball* 1995b). After a normal delivery, ovarian activity can be resumed from the 2nd week after parturition. Dystocia and genital post partum problems can also affect the calving to conception interval.

It seems likely that also the induction of parturition could influence the onset of ovarian cyclicity after calving. It was reported that after

the induction of parturition with dexamethasone in primiparous dairy cows, the ovarian cyclicity started soon after calving (*Kask et al.* 2000a), while after the induction of parturition with $PGF_{2\alpha}$, ovarian cycles did not resume until 40 days after parturition at the earliest (*Kask et al.* 2000b).

One of the most successful treatments to induce the resumption of ovarian activity in post partum dairy cows is the administration of progesterone. The theory behind progesterone administration relies partially on the ability of this hormone to initiate regular pulses of LH similar to those in the mid-luteal phase. Very often in anoestrous situations LH, if secreted at all, is released in a random, disorganized pattern of pulses. Once appropriate pulses of LH are released, any Graafian follicles present will grow in the ovary and upon progesterone withdrawal a normal follicular phase will continue (*Meredith* 1995).

Watson & Munro (1984) reported a significant increase of plasma progesterone in ovariectomized cows after administration of 10 and 500 μg ACTH-analogue (tetracosactid). In addition *Bolaños et al.* (1997) demonstrated a significant increase of plasma progesterone in ovariectomized zebu cows after the administration of 6 μg of tetracosactid.

The aim of this study was to evaluate the effect of an early post partum treatment with tetracosactid on the synthesis and release of steroids (cortisol and progesterone) from the adrenals and on the $PGF_{2\alpha}$ release, evaluating, in turn, the effect on the resumption of ovarian cyclicity in primiparous dairy cows after induction of parturition with $PGF_{2\alpha}$.

Materials and methods

The study was conducted in 3 primiparous cows. Two Swedish Red and White breed cows (A and B) and 1 Swedish Friesian breed cow (C) were brought to the Department of Obstetrics and Gynaecology about one month before the date of expected parturition. The cows were housed in individual pens and fed ad libitum according to Swedish standard (*Spörndly* 1993).

At 268 days of pregnancy the cows were treated twice with 25 mg i.m. $PGF_{2\alpha}$ (Dinolytic, Pharmacia & Upjohn, Puurs, Belgium) 24 h apart.

Starting on day 12 post partum (PP) the cows were treated with 500 μg i.m. of tetracosactid (Synachten®, Novartis Pharma, Basel, Switzerland) every 6 h six times.

The effects of the treatment were evaluated immediately after the administration of the drug to detect any changes in hormonal plasma concentrations and during the 8 weeks after parturition to detect any possible effect on the resumption of ovarian activity after calving.

Short time effects

Hormonal analysis was performed to evaluate changes in progesterone (P4), cortisol (C) and 15-ketodihydro-$PGF_{2\alpha}$ (the main metabolite of $PGF_{2\alpha}$) (PG-metabolite) in jugular vein blood samples.

Blood samples were collected every 30 min (-60, -30, -0, 30, 60, 90 min) 3 times before and 3 times after the 1st and the 6th injections of tetracosactid.

Effect on ovarian cyclicity

The evaluation of the effects of tetracosactid administration on the resumption of ovarian cyclicity was carried out by means of clinical examination, ultrasound examination and hormonal analyses.

Clinical evaluation was made twice daily from parturition to day 56 after calving to assess shed of placenta, vaginal discharge and signs of oestrus. The fetal membranes were defined as retained if they were not expelled within 24 h after parturition (*Arthur* 1979, *Bekana et al.* 1994, *Mollo et al.* 1997).

Ultrasound examination of the ovaries was performed 3 times weekly (Tuesday, Friday, Sunday) starting on day 9-10 until day 55-56 PP. The ultrasound equipment used was a real time B-mode linear array scanner (Aloka SSD-210 DXII, Japan) with a 7.5 MHz transducer. The instrument was supplied with standard video system and the images were recorded on videotapes. The equipment had also an image freezer facility with electronic callipers for taking measurements. Follicles were counted and classified in 2 main categories on the basis of their diameter:

Class 1 = follicles <10 mm of diameter;
Class 2 = follicles ≥10 mm of diameter.

Number and life-span of CL were also evaluated.

Hormonal analysis was carried out to evaluate P4 and PG-metabolite profiles from day 4 to day 56 after parturition in jugular vein blood samples. Blood samples were collected twice daily (morning and evening) for PG-metabolite analysis and once a day (only in the morning) for P4 analysis. The samples were taken into heparinised tubes (Terumo Europe, Leuven, Belgium) and immediately centrifuged. Plasma was removed and stored at –20 °C until hormone analysis was performed.

Progesterone was determined by a Coat-A-Count DPC kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Serial dilutions of bovine plasma with high concentrations of P4 produced inhibition curves parallel to the standard curve. The sensitivity of the assay was 0.1 nmol/l. According to the manufacturer the antiserum shows no cross-reactivity with C. The intra-assay coefficients of variation for 3 control samples (low, 2.3 nmol/l; medium 25.7 nmol/l and high 74.2 nmol/l) assayed in duplicates in 20 assays were 10.6%, 4.7% and 7.1% respectively. The corresponding inter-assay coefficients of variation were 8.9%, 10.1% and 13.3% (*Bolaños et al.* 1997).

Cortisol was determined by a Coat-A-Count, solid phase radio immunoassay kit (Diagnostic Products Corporation). The detection limit of the assay was 5.5 nmol/l. According to the manufacturer the antiserum shows low cross-reactivity with P4 (0.15%). Quality control samples containing endogenous cortisol were assayed in duplicates at the beginning and end of each assay. The intra-assay coefficient of variation was between 2.2% and 6.3%. The inter-assay coefficient of variation was between 3.8% and 5.2% (*Bolaños et al.* 1997).

The plasma samples were analysed for concentration of PG-metabolite, according to *Granström & Kindahl* (1982). The relative cross-reaction of the antibody raised against 15-ketodihydro-$PGF_{2\alpha}$ were 16% with 15-keto-$PGF_{2\alpha}$, 4% with 13,14-dihydro-$PGF_{2\alpha}$ and 1.7% with the corresponding metabolite of PGE_2. The lower limit of detection of the assay was 30 pmol/l for 0.5 ml plasma. All high levels were estimated, but for better interpretation, an upper limit was set at 3000 pmol/l in the figures. The intra-assay coefficient of variation varied between 6.6% and 11.7% at different ranges of standard curve.

The onset of ovarian cyclicity was demonstrated by ultrasound detection of a CL and P4 levels ≥3 nmol/l. Ovarian cyclicity was considered normal when P4 values ≥3 nmol/l were found for at least one week.

Progesterone, C and PG-metabolite plasma concentrations before and after the 1st and the 6th tetracosactid administrations were submitted to a paired samples t-test analysis. Differences between P4, C and PG-metabolite after the 1st and the 6th administration of tetracosactid were analysed by means of a 2-way ANOVA (administration and time post-injection).

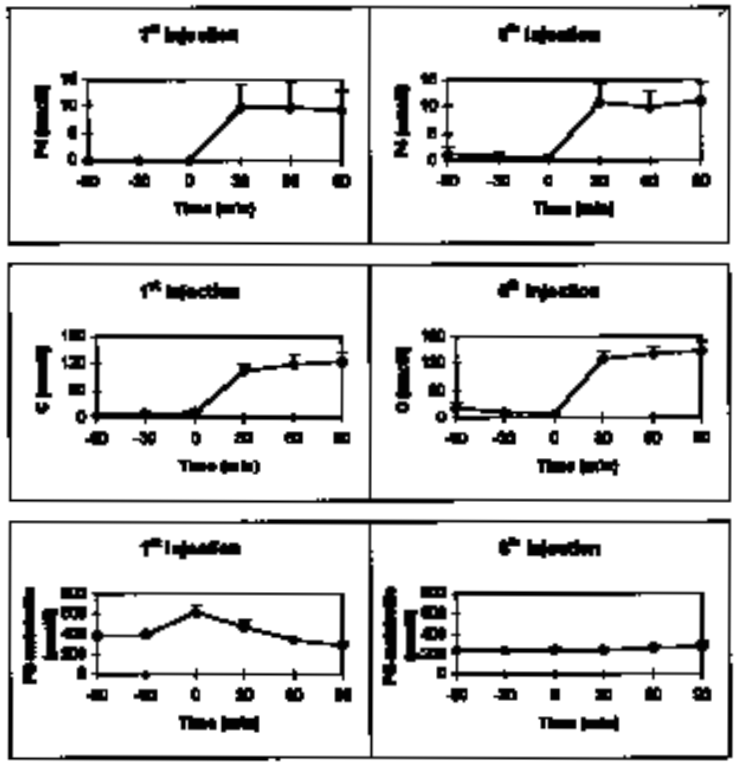

Figure 1: P4, C and PG-metabolite plasma concentrations (mean + SEM) around the 1st and the 6th administrations of tetracosactid.

Results

The cows had parturition at a mean of 52 h (cow A = 48 h, cow B = 51 h, cow C = 57 h) after the 1st injection of $PGF_{2\alpha}$.

Before treatment

All the 3 cows retained the placenta after induction of parturition with $PGF_{2\alpha}$. They shed the placenta on days 9, 12 and 10, respectively. The ultrasound examination of the ovaries performed before the beginning of treatment showed in all 3 cows the presence of only Class 1 follicles.

From day 4 until day 11 after parturition P4 values were less than 0.5 nmol/l. On day 4 PG-metabolite values were >5475 pmol/l, on day 8 they fell down to <500 pmol/l and until day 11 they were fluctuating from 399 to 722 pmol/l, in all cows.

Around treatment

The mean P4 concentrations in plasma samples collected from 60 min before the 1st injection of tetracosactid were zero in all the cows. During the 90 min after the 1st injection the average (means + SEM) values were 18.0+0.9, 3.8+0.2 and 7.1+0.8 nmol/l, in 3 cows respectively. From 60 min before the 6th administration the

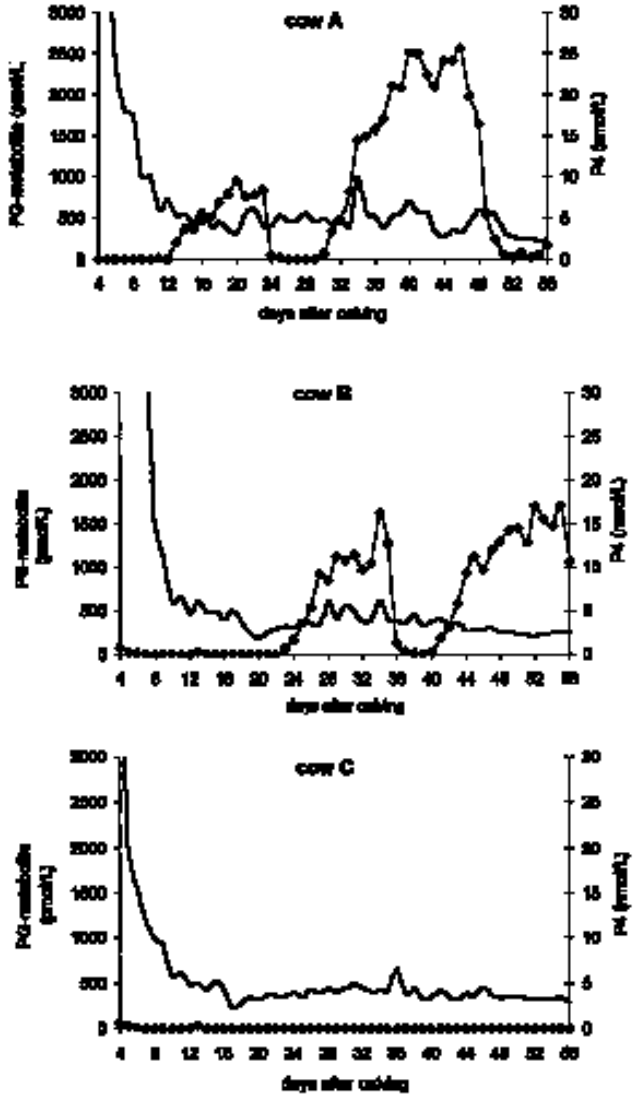

Figure 2: P4 (-◆-) and PG-metabolite (—) plasma levels during 56 days after calving in cows A, B and C.

mean values were 2.3+0.6, 0, 0 nmol/l, respectively, and after the 6th injection they were 17.0+0.7, 6.2+0.4, 8.4+0.6 nmol/l. Before the 1st injection of tetracosactid, the mean values for cortisol were 14.0+4.4, 7.0+2.6, 5.3+0.7 nmol/l, respectively and after the same injection they rose to 149.7+11.1, 88.3+4.3, 107.7+4.6 nmol/l. The mean values, before the 6th injection, were 25.7+8.6, 8.7+1.4, 8.7+1.2 nmol/l and after they were 162.7+5.9, 103.7+4.5,

152.0+7.2 nmol/l, respectively. About prostaglandins, the mean values in the 3 cows before and after the first injection, were 483.7+87.4, 479.7+111.1, 423.0+32.8 pmol/l and then 340.0+48.2, 440.7+80.3, 313.7+34.9 pmol/l, respectively. Before and after the 6th administration they were 256.0+6.3, 212.0+9.3, 244.0+15.3 pmol/l and 310.3+22.4, 232.7+7.2, 241.0+2.3 pmol/l, respectively. The mean values (+ SEM) for the 3 parameters are presented in Fig. 1.

Significant differences emerged from the results obtained before and after the 1st administration of tetracosactid for P4 ($t = -4.7$; $p<0.01$) and C ($t = -12.6$; $p<0.01$). The comparison of the results obtained before and after the 6th administration of tetracosactid showed significant differences for P4 ($t= -7.5$; $p<0.01$) and for C ($t=-13.8$; $p<0.01$). PG-metabolite did not show any statistical difference before and after both the 1st and the 6th administrations ($p>0.05$).

The ANOVA test for plasma concentrations of P4 ($F=0.085$; $p>0.05$ and $F=0.006$; $p>0.05$) and C ($F=2.600$; $p>0.05$ and $F=0.615$; $p>0.05$) showed no significant differences after the 1st and the 6th administrations.

Post treatment

Signs of oestrus were seen on day 28 and on day 53 after parturition in cow A, and on day 39 PP in cow B. In cow C, no signs of oestrus were seen during the whole period of observation.

The ultrasound examination showed different number of Class 1 follicles in both the ovaries during the whole period of observation in the three cows. In cow A, the first CL was seen from day 18 to 27 and the 2nd CL from day 34 to 51, with 4 follicular waves during the whole period of observation. In cow B, ultrasound examination showed the 1st CL from day 28 to 37 and the 2nd from day 42 to 56, with 4 follicular waves. In cow C no CLs were detected until day 56, even if follicular waves were seen in both ovaries on day 17-24 and 24-33. Then, a follicle-like structure persisted until the end of the study, reaching a maximum diameter of 18 mm. Cow A showed P4 levels between 3.5 and 9.4 nmol/l from day 14 until day 23 after parturition and then, P4 dropped to <0.7 nmol/l from day 24 to day 30 (Fig. 2). Then, P4 levels rose to values between 3.4 and 25.6 nmol/l from day 31 to day 49 and dropped to <2.4 nmol/l from day 50 to the end of the study. In cow B from day 14 to day 24 the P4 levels were <1.7 nmol/l. From day 25 they rose to values of P4 between 3.6 and 16.2 until day 35 and then they dropped to <1.9 nmol/l in the next 6 days. Then the P4 level rose again to values between 3.1 and 16.9 nmol/l from day 42 to the end of the study. In cow C the levels of P4 remained around zero during the whole period of observation.

PG-metabolite values ranged in all the three cows between 654 and 181 pmol/l from day 14 to 56 after parturition (Fig. 2).

Discussion

Most of the previous studies evaluated the effect of the administration of tetracosactid only like a stressor (*Watson & Munro* 1984, *Alam et al.* 1986, *Bolaños et al.* 1997, *Torres et al.* 1997), but this is the first report in which the possible role of the drug as a stimulus for steroid output and consequently to stimulate the onset of ovarian cyclicity was studied.

The evaluation of the effects immediately after the administration of tetracosactid showed that the treatment was able to induce a statistically significant ($p<0.01$) increase in C and P4 plasma concentration after both the 1st and the 6th injections, in all the cows. The 1st and the 6th administrations were used to evaluate the refractoriness of the adrenals to multiple injections, but no statistically significant differences ($p>0.05$) were seen. Because of the lack of luteal tissue at the beginning of the tetracosactid administration, P4 should most likely be

considered to be released from the adrenals. The differences observed amongst cows in the magnitude of P4 output is in agreement with *Watson & Munro* (1984) that reported a large individual variation in response to tetracosactid administration in cows.

No changes in PG-metabolite plasma concentrations were seen after both the 1st and the 6th injections of tetracosactid. This is in contrast with previous study in pig (*Mwanza et al.* 2000a,b) in which a large increase in PG-metabolite was seen after a treatment with a large dose (10 times higher) of Synachten® Depot (longacting tetracosactid) injected intravenously. *Mwanza et al.* (2000a) are demonstrating that a refractoriness is induced if tetracosactid is injected in pigs several times and that the only response in PG-metabolite levels is seen after the first injection. In a study by *Madej et al.* (2000), a PG-release was seen also after tetracosactid and irrespective of 10 or 1 μg/kg bw. The most likely explanation for this difference between the pig and cow is that our study is performed in the post partum period where large synthesis of prostaglandins already is occurring.

It is more difficult to evaluate the effects of treatment on the resumption of ovarian cyclicity. Ultrasound examination showed that Class 1 follicles were present on days 9-10 in all the three cows and no luteal tissue was detected before the administration of tetracosactid (days 12 and 13). The first CL was seen 5 days after the end of the treatment (day 18 PP) in cow A, and 15 days after the tetracosactid administration (day 28 PP) in cow B and, in both cases, it was followed by a normal ovarian cyclicity. No CLs were observed during the whole period of study in cow C, even if follicular waves were seen until day 33 post partum. Progesterone profiles confirmed the ultrasound observations in the three cows.

The clinical examination before the treatment revealed that cow C had fever during the first week after calving and that food intake was less than in the other animals. This cow of Swedish Friesian breed had higher milk yield compared with the others (30 vs 15 l/day).

Considering that *Kask et al.* (2000b), reported that no ovulation were seen during the first 40 days PP in cows after induction of parturition with $PGF_{2\alpha}$, it could be anticipated that the tetracosactid administration could influence the onset of ovarian cyclicity (18 and 28 days PP). Nevertheless, it seems difficult to evaluate in which way the administration with tetracosactid could have affected the onset of ovarian cyclicity because of the different pattern of response to the treatment. In conclusion, we consider that the possible influence of tetracosactid administration on the onset of ovarian cyclicity in post partum primiparous dairy cows should be an interesting subject for further studies.

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Sammanfattning

Effekt av tetrakosaktid på äggstocksaktiviteten postpartalt hos kor efter förlossningsinduktion med $PGF_{2\alpha}$.

Förlossning och kvarbliven efterbörd inducerades med $PGF_{2\alpha}$ hos tre mjölkkvigor. Med start från dag 12 i postpartumperioden behandlades korna med 500 μg i.m. med en ACTH-analog (tetrakosaktid) var 6:e timme och totalt 6 gånger. Förändringar i plasmanivåer av kortisol, progesteron och 15-ketodihydro-$PGF_{2\alpha}$ utvärderades omedelbart efter behandling. Återupptagandet av äggstocksaktivitet studerades med såväl klinisk och ultraljudsundersökning som med progesteron och 15-ketodihydro-$PGF_{2\alpha}$ analyser under 56 dagar efter förlossningen. Behandlingen med tetrakosaktid orsakade hos alla djur en statistisk signifikant ($p<0.01$) likartad ökning i kortisol och progesteron efter både den första och den sjätte injektionen. Inga förändringar i prostaglandinmetaboliten kunde spåras. Den första gulkroppen sågs dag 18 efter förlossningen hos ko A respektive dag 28 hos ko B. Hos båda djuren följde en normal äggstocksaktivitet. Ingen gulkroppsutveckling kunde ses hos ko C. Dessa kliniska och ultraljudsmässiga observationer konfirmerades av progesteronprofiler postpartalt. Det synes som om framför allt progesteronfrisättningen orsakad av tetrakosaktid kan vara en stimulans för äggstockarna eftersom två av tre djur ovulerade tidigare än förväntat. Detta tyder vidare på att en påverkan på stressystemet (binjurarna) kan ha en positiv effekt på äggstocksaktivitet. Eftersom djuren svarade olika bör fortsatta studier genomföras.

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