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Pharmacokinetic analysis during intravaginal progesterone-releasing devices (PRID®/CIDR®) application in the mare

Oliver Pohl[1], Samuel Buff[2], Francois Garnier[2], Pierre Guérin[2] and Anne-Cécile Lefranc[2]

Institute of Cancer Research, Department of Medicine I, Medical University Vienna, Austria[1] and Ecole Nationale Vétérinaire de Lyon, Equine Department, Biology and Pathology of Reproduction, Marcy l'Etoile, France[2]

Summary

Exogenous administration of synthetic and natural progestin is commonly used in the mare during the annual transitional period to hasten the onset of the breeding season, as well as during the breeding season to suppress or synchronise oestrus and to maintain high blood progesterone concentration during pregnancy. Various routes of administrations were developed, one being the intra-vaginal insertion of a device, which allows continuous and prolonged absorption of progesterone. With the recent ban of oestradiol in cattle, "progestin-alone" devices are the only products available in the cattle industry, two of which are PRID® and CIDR® (1,38g). The aim of this study was to investigate the pharmacokinetic parameters C_{max}, t_{max}, AUCs and t½ from individual serum in mares with initially low progesterone serum concentration during an 8-day intra-vaginal administration of these two products. Similarities were found between the two devices with an adequate maintenance of high blood progesterone during administration. For both devices, the serum progesterone concentration pattern showed a rapid rise with a similar peak (C_{max}(CIDR®) = 17.8 nmol/L and C_{max}(PRID®) = 19.9 nmol/L) followed by a plateau and a rapid drop at device removal with concentrations < 3 nmol/L reached in less than 2 hours and an initial half-life (t½) on the 0 to 120 minutes interval around 35 minutes. The AUC was found within the same range for both devices (1958 nmol/L/h and 2468 nmol/L/h for CIDR® and PRID®, respectively). It may hence be hypothesised that both intra-vaginal "progestin-alone" devices, CIDR® and PRID®, could be useful in the mare to enhance the first oestrous cycle during the transition period or to suppress oestrus or help in the maintenance of pregnancy in individual mares during the breeding season.

Keywords: mare, progesterone, intra-vaginal device, pharmacokinetic

Pharmakokinetische Untersuchungen während der Anwendung intravaginaler Progesteron-freisetzender Applikationssysteme (CIDR®/PRID®) beim Pferd

Die Behandlung von Stuten mit natürlichen oder synthetischen Progestagenen gehört zu den gynäkologischen Standardbehandlungen. Während der anöstrischen Periode des Geschlechtszyklus der Stute erlaubt diese Behandlung einen früheren Zuchteinsatz in der folgenden Saison. Während der Zuchtsaison kann durch die Progestagengabe eine Zyklushemmung oder Synchronisation erreicht werden oder eine Trächtigkeit hormonell unterstützt werden. Kommerzielle Progestagenprodukte existieren in verschiedenen Arzneimittelformen – ihr Einsatz als intravaginales Medizinprodukt erlaubt die kontinuierliche Substanzabgabe über längere Zeiträume. Seit dem Verbot der Östradiolbehandlung in der Mastindustrie stehen intra-vaginale Progesteronformulierungen wie zum Beispiel PRID® und CIDR® (1,38g) nur noch für die Rindermast zur Verfügung. Das Ziel dieser Studie war die Charakterisierung und der Vergleich pharmakokinetischer Parameter wie maximale Serumkonzentration (C_{max}), Zeit bis zum Erreichen der maximalen Serumkonzentration (t_{max}), Flächeninhalt unter der Serum-Konzentrations-Zeit-Kurve (AUC) und die Serumhalbwertzeit (t½) während einer achttägigen Behandlungsphase mit PRID® und CIDR® bei Stuten. Das Serum stammte von einzelnen anöstrischen Stuten mit niedrigen Progesteronserumkonzentrationen bei Behandlungsbeginn. Beide Produkte zeigten vergleichbare pharmakokinetische Eigenschaften und erreichten hinreichend hohe Serumkonzentrationen während der Behandlungsphase. Das Serumprofil zeigte einen schnellen Anstieg auf maximale Serumkonzentrationen (C_{max}(CIDR®) = 17.8 nmol/L und C_{max}(PRID®) = 19.9 nmol/L) gefolgt von einer Plateauphase und einem schnellen Konzentrationsabfall nach Behandlungsende. Konzentrationen unter 3 nmol/L wurden in weniger als 2 Stunden erreicht, die Halbwertzeit während der ersten 120 Minuten nach Behandlungs-ende (t½) betrug zirka 35 Minuten. Auch die AUCs beider Produkte befanden sich in ähnlichen Bereichen (1958 nmol/L/h und 2468 nmol/L/h für respektive CIDR® und PRID®). Beide intra-vaginale Progesteronformulierungen (CIDR® and PRID®) könnten aufgrund ihrer vergleichbaren pharmakokinetischen Eigenschaften nützliche Hilfsmittel zum früheren Zuchteinsatz der Stuten nach der Zykluspause sein. In gleichem Maße wäre ihr Einsatz zur Zyklushemmung, Synchronisation oder hormonellen Unterstützung der Trächtigkeit während der Zuchtsaison denkbar.

Schlüsselwörter: Stute, Progesteron, intra-vaginal Formulierung, Pharmakokinetik

Introduction

The economic challenge of producing foals early in the year resulted in the use of commercial progestin products during the transition period to hasten the onset of the breeding season (*Squires* et al. 1979). Other indications for exogenous administration of synthetic and natural progestin were developed for use during the breeding season:

- to suppress oestrus in mares exhibiting excessive and unwanted oestrous behaviour,
- to maintain pregnancy in mares presenting a history of early pregnancy losses. Treatment is initiated at the time of pregnancy diagnosis (around 14 days of gestation) and continued until 120 - 150 days of gestation, i.e. until the progesterone secretion is taken over by the placenta,

- to synchronise oestrus between mares in embryo transfer programs or in artificial insemination protocols to minimise the number of semen collections. The underlying mechanism is the inhibitory effect of progesterone on the hypothalamic GnRH release. At the end of the treatment, the progesterone level decreases and leads to an increase in LH pulse frequency allowing ovulation of the dominant follicle (*Squires* 1993),
- to insure high blood progesterone concentration in recipient mares undergoing an embryo transfer.

Various routes of administration were evaluated in the mare. The intra-muscular route requires daily injections of a progesterone-in-oil product, which frequently causes local pain and may lead to the formation of an abscess (*Evans* et al. 1982, *Taylor* et al. 1982, *Burns* et al. 1993). The recently commercialised slow-releasing formulations, which are exclusively available in the United States, allow high blood progesterone concentration for approximately 7 days with a single injection (*Burns* et al. 2008a and 2008b).

The oral route uses progesterone micronized in oil or with fatty foods to allow absorption of the product. The oily allyl-trenbolone (altrenogest) solution (Regumate®, Intervet S. A., Beaucouzé, France), thoroughly studied in the mare (*Allen* et al. 1980, *Webel* and *Squires* 1982, *Squires* et al. 1992, *Bruemmer* et al. 2000), requires daily administration and manipulation precautions due to its local skin absorption and androgens effects.

The intra-vaginal route employs devices originally developed for the ovine and bovine industries to synchronise and/or induce oestrus (*Munro* 1990, *Broadbent* et al. 1993, *Uehlinger* et al. 1995, *Galvao* et al. 2004, *Stevenson* et al. 2006): sponges (*Dinger* et al. 1981), PRID® with oestradiol (*Rutten* et al. 1986, *Ataman* et al. 2000, *Newcombe* 2002), CIDR® (*Jöchle* et al. 1991, *Arbeiter* et al. 1994, *Newcombe* and *Wilson* 1997, *Handler* et al. 1999, *Foglia* et al. 1999). A device developed for mares was also made available in Australia under the name of Cue-Mare® (*Grimmett* et al. 2002). In all these products, progesterone is released continuously and absorbed through the vaginal mucosa. Most of the published referred to the combined oestradiol-progesterone PRID® device and the CIDR® (1,99g). For these latter, plasma progesterone concentrations seem to follow a similar pattern in ovariectomised cows (*Munro* 1987) and the capacity of progesterone release from the intra-vaginal device appears to be the main factor limiting vaginal uptake of progesterone (*Uehlinger* et al. 1995). In the mare, *Handler* et al. (2006) investigated the effects of reproductive status and plasma progesterone concentrations of mares at PRID® (progesterone-alone device) insertion on the progesterone plasma concentration: in cyclic mares, the plasma progesterone concentrations at insertion of PRID® seemed to be more important for the efficacy of the treatment than the assignment to oestrous cycle stages.

To our knowledge, no comparison in the progesterone serum pattern was studied in the mare between the two progesterone-alone devices, PRID® and CIDR® (1,38g). The purpose of this study was to investigate and compare pharmacokinetic parameters (C_{max}, t_{max}, AUCs and t½) from individual serum in mares with initially low progesterone serum concentration during an 8-day intra-vaginal administration of the two devices, PRID® and CIDR® (1,38g).

Material and Methods

Animals

A health examination was performed on normal cycling mares, weighing 500-650 kg and aged 5 to 8 years, to insure satisfactory health status. Ultrasonographic examination of the genital tract was regularly performed and all mares presenting a corpus luteum older than 5 days received simultaneously an intramuscular injection of 250-375 g of the prostaglandin F2 analogue, cloprostenol (Estrumate, Schering-Plough Vétérinaire, Levallois-Perret Cedex, France) to induce oestrus. Mares were examined three days after the injection and seven of them were enrolled in experience 1, while presenting no trace of corpus luteum, no follicle ≥ 20 mm and a serum progesterone concentration < 3 nmol/L. Mares were allocated into two separate groups according to a pre-established randomisation list and were housed in boxes under the same conditions. In experience 2, two mares of each group were used again to determine more precisely the progesterone fall pattern at the time of device removal.

Intra-vaginal progesterone releasing devices

After emptying the rectum of the mare with a lubricated glove, a rectal examination of the genital tract was performed. The tail was bandaged and tied up out of the way before the vulva and perineum were washed thoroughly with an antiseptic solution, rinsed with running water and dried with disposable paper. The PRID® device (Ceva Santé Animale, Libourne, France), containing 1.55g progesterone in an inert spiral silicone elastomer coil, and the CIDR® device (Pfizer Santé Animale, Paris, France), containing 1,38 g progesterone in molded silicone over a flexible nylon "Y-shaped" spine, were used. In experience 1, both devices were placed into the vagina of randomised mares and left in place for 8 days. Daily examination was performed to check the presence of the string at the vulva lips and to note any vaginal discharge. Devices were removed at the end of the application period by pulling the string hanging out of the vulva.

Sampling protocol

Jugular vein blood samples (10 mL) were collected into plastic blood tubes containing no anticoagulant, allowed to clot and separated by centrifugation at 4000xg for 10 minutes at room temperature. Serum progesterone concentrations were determined with an Immulite 2000 Analyser using the Progesterone Test L2KPW2, a solid-phase competitive chemiluminescent enzyme immunoassay (cf note infra après Acknowledgment) validated in horse serum. The limit of quantification of the test is 0.64 nmol/L, the repeatability range between 17% (1.46 nmol/L) and 7% (68.7 nmol/L) and the reproducibility range between 28% (1.46 nmol/L) and 9.5% (68.7 nmol/L).

In the first experiment, samples were collected at 0, 1, 3, 6, 9 and 18 hours after device insertion; thereafter a daily blood

sample was taken for 8 days. On the last day (192 hours), the device was gently removed by pulling the string and a vaginoscopic examination was performed to note any local inflammation and/or liquid accumulation. Blood samples were then collected 1, 3, 6, 9, 12, 24 and 48 hours after device removal. On the second experiment, samples were collected 0, 10 20, 30, 40, 50 minutes and 1, 2, 3 and 4 hours after device removal.

Pharmacokinetic analysis

The pharmacokinetic evaluation was performed using the validated software WinNonLin V 4.1 (Pharsight Corporation, Mountain View, CA 94040, USA) according to a non-compartmental analysis. The average (mean) pharmacokinetic parameters were calculated from the serum concentrations of each individual mare. Standard deviation (S.D.) and coefficient of variation (C.V.) were calculated to assess inter-individual variability.

The following rules were applied for calculation of the pharmacokinetic characteristics: at time points in the lag-time between time zero and the first quantifiable concentration, a concentration below the lower limit of quantification was set to zero for mean and AUC calculations; a concentration below the lower limit of quantification between two quantifiable concentrations was set to zero for mean calculation and excluded from AUC calculation; after the last quantifiable concentration, a concentration below the lower limit of quantification was set to zero for mean calculation and excluded from AUC calculation.

The following pharmacokinetic parameters were calculated:
- C_{max} (maximum concentration) was read directly from the concentration-time plots;
- t_{max} (time to maximum concentration) was read directly from the concentration-time plots;
- t½ (initial half-life) was estimated on the first 120 minutes of elimination
- AUC0-t (area under the curve from 0 hour to the time point of the last quantifiable concentration) was calculated according to the linear up/log down trapezoidal method and using nominal sampling times;
- lz (elimination rate constant) was determined by the log linear regression obtained on at least the three last quantifiable concentrations. The correlation coefficient (r2) for the goodness of the fit of the regression line through the data points had to be ≥ 0.85, for the value to be considered reliable;
- AUC0-inf (area under the curve from time 0 to infinity) was calculated as the sum of AUC0-t and AUCt-inf, where AUCt-inf = Ct / lz measured concentration at the last quantifiable concentration. AUC0-inf was considered less reliable when lz was less reliable and in addition, if the extrapolation to infinity from the last measurable data point constituted more than 20% of the total AUC0-inf.

Results

The devices remained in place for the 8-day period in all mares, with no sign of discomfort. In the CIDR® group one device was removed 18 hours post-insertion due to unrelated colics in the mare but blood samples were taken under the same time schedule after removal as described in Material and Methods. A vaginal secretion was visible with both devices during administration and disappeared within 48 hours of device removal. All mares showed mild vaginitis, with isolation of Streptococcus equi subsp. zooepidemicus.

All serum concentrations at the time of device insertion were below the lower limit of quantification. Individual progesterone serum concentrations during and after CIDR® and PRID® administration are presented in Figures 1 and 2 respectively.

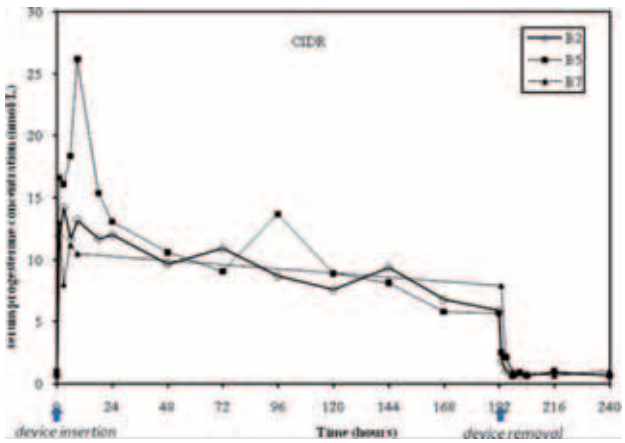

Fig. 1 Individual progesterone serum concentration during CIDR® administration and after device removal.
Individuelle Progesteron Serumkonzentration während der CIDR®-Anwendung nach der Entfernung des Trägers.

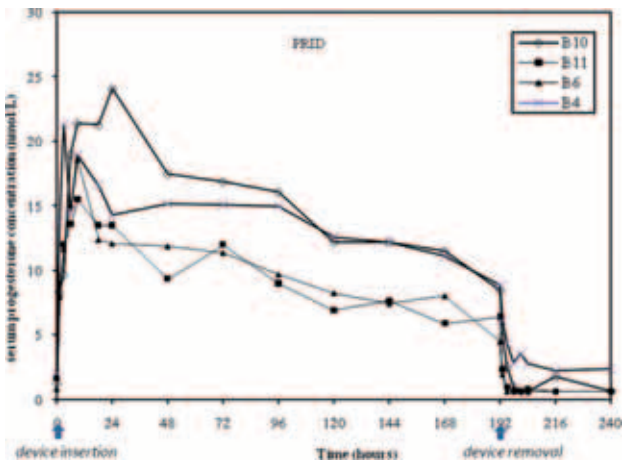

Fig. 2 Individual progesterone serum concentration during PRID® administration and after device removal.
Individuelle Progesteron Serumkonzentration während der PRID®-Anwendung nach Entfernung des Trägers.

After intra-vaginal device insertion, progesterone was rapidly absorbed with time to maximum concentration (t_{max}) reached between 1-9 hours and 3-9 hours with the CIDR® and the PRID® respectively. For this latter, one mare had a t_{max} at 24 hour post-insertion, but with a concentration very closed to the high value obtained at 9 hours post-insertion. The mean serum progesterone concentration during CIDR® and PRID® administration is presented in Figure 3. The mean (S.D.) pharmacokinetic parameters during both devices administration are presented in Table 1. The mean peak progesterone serum

concentration (C_{max}) was 17.8 nmol/L and 19.9 nmol/L with the CIDR® and PRID® devices, respectively. The AUC was 1958 nmol/L/h and 2468 nmol/L/h for CIDR® and PRID® respectively. The inter-individual variation in progesterone serum concentration during CIDR® and PRID® administration evaluated by % C.V. (Coefficient of Variation) ranged from 18-51 and 13-38% respectively, i.e. a slightly larger variability was observed with the CIDR® device. After device removal, the progesterone concentration decreased rapidly reaching values below 3 nmol/L between 30-60 min with the CIDR® and 10-20 min or 60-120 min with the PRID® (Figure 4). The initial half-life (t½) calculated on the interval period from 0-120 minutes was found between 36.6 and 37.3 minutes after CIDR® removal and between 35.3 and 38.3 minutes after PRID® removal.

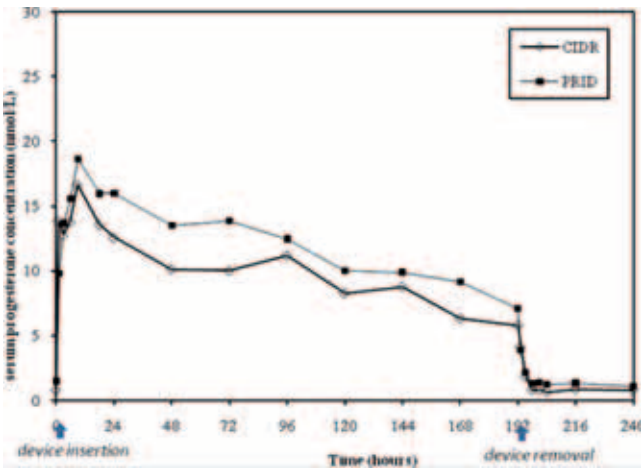

Fig. 3 Mean serum progesterone concentration during CIDR® and PRID® administration.
Mittlere Progesteron-Serumkonzentrationen während der CIDR®- und PRID®-Behandlung.

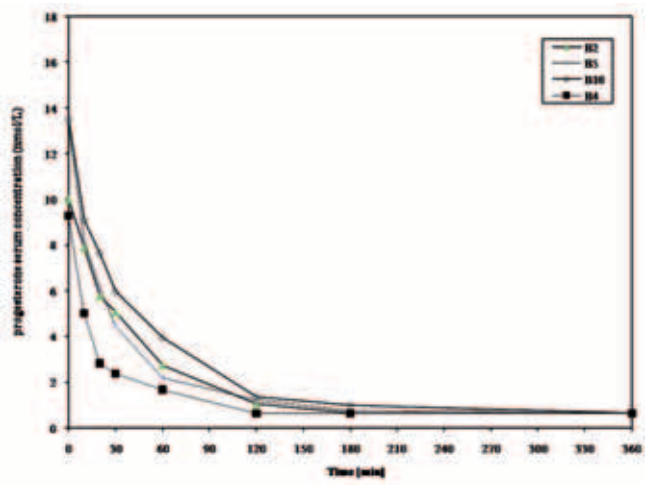

Fig. 4 Individual progesterone serum concentration after CIDR® (B2, B5) and PRID® (B4, B10) removal (T0).
Individuelle Progesteron-Serumkonzentration nach CIDR® (B2, B5) und PRID®-Entfernung (T0).

Discussion

The pharmacokinetics parameters for the intra-vaginal "progesterone-alone" devices PRID® and CIDR® showed high similarities and an adequate maintenance of high blood progesterone during administration. For both products, the progesterone serum concentration followed the bellowed pattern: a rapid rise at insertion, with a similar peak serum concentration reached within 9 hours of insertion with both devices (17.8 nmol/L and 19.9 nmol/L with the CIDR® and PRID®, respectively), a plateau during the 8-day administration with an AUC within the same range (1958 nmol/L/h and 2468 nmol/L/h for CIDR® and PRID®, respectively) and a rapid drop at removal with progesterone concentrations below 3 nmol/L in less than 2 hours and an initial half-life (t½) on the 0 to 120 minutes interval around 35 minutes for both devices.

Intra-vaginal progestin application is a non-invasive and non-painful procedure that allows rapid and prolonged product absorption. In comparison to the intramuscular and oral administration routes, it requires minimal manipulation with a two-step procedure consisting of the insertion and removal of the device. Still, it is not the most common route employed in the mare, mainly because of the vaginal discharge often present during administration. This physiologic response to a foreign device is responsible for a mild vaginitis, which has been proven not to interfere with fertility. Due to the little effect of progesterone on follicular development, oestradiol is often combined to progestin for its stronger inhibitory effect on FSH secretion. The recent European ban of oestradiol in products marketed for "food chain" implied the commercialisation of oestradiol-free progestin products. Hence, the oestradiol benzoate capsule of the intra-vaginal device PRID®-2MM, renamed Pridoestrol®, was removed to create the new PRID® device available for oestrus synchronisation and induction in cattle. In France, the Pridoestrol® device is the only intra-vaginal device that has a market authorisation for use in mares. This year, the CIDR® intra-vaginal device obtained a market authorisation for use in cattle. In Germany, both CIDR® and PRID® have a market authorisation for use in cattle but not for use in horses. It can hence be speculated that both the PRID® and the CIDR® progesterone-alone devices could be made

Table 1 Mean (S.D.) pharmacokinetic parameters during CIDR® and PRID® administration. / *Mittlere pharmakokinetische Parameter während der CIDR®- und PRID®-Behandlung.*

	CIDR®		PRID®	
	Mean (S.D.)	% C.V.	Mean (S.D.)	% C.V.
$C_{max\ (nmol/L)}$	17.8 (7.3)	41	19.9 (3.7)	19
$T_{max\ (h)}$	4.3 (4.2)	98	11.3 (9.0)	80
$AUC_{0\text{-}240h\ (nmol/L/h)}$	1892 (131)	7	2364 (587)	25
$AUC_{0\text{-}inf\ (nmol/L/h)}$	1958 (116)	6	2468 (649)	26

available in the future in the mare either to enhance the first oestrous cycle during the transition period and to suppress oestrus, or to help in the maintenance of pregnancy in individual mares during the breeding season. Another economical potential would be the use of such devices in embryo transfer programs to synchronise donor and recipient mares, as well as to insure high blood progesterone concentration in the recipient mares. Further studies are required to compare the degree of oestrous cycle synchrony with both devices, as well as the impact on heat induction early in the breeding season.

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Dr. Anne-Cécile Lefranc
DVM, MSc, PhD, Dipl.ECAR
Assistant Professor of Theriogenoloy
Université de Lyon
Ecole Nationale Vétérinaire de Lyon
Equine Department, Biology and Pathology of Reproduction
69280 Marcy l'Etoile
France
ac.lefranc@vet-lyon.fr