

The effect of deslorelin acetate on the oestrous cycle of female guinea pigs

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ABSTRACT: Deslorelin acetate, a GnRH agonist, is commonly used to prevent folliculogenesis in several species. However, little is known of the effect of deslorelin acetate implants on the oestrous cycle of female guinea pigs. Fifteen intact healthy female guinea pigs were investigated in this study. Signs of sexual behaviour, the presence of a vaginal membrane along with plasma concentrations of oestradiol (E_2) and progesterone (P_4), were monitored during two consecutive oestrous cycles. At the beginning of the third oestrous cycle each guinea pig was administered an implant of the GnRH analogue, deslorelin acetate, (4.7 mg). When compared to the untreated state, deslorelin implantation was associated with altered signs of oestrus. The average time to opening of the vaginal membrane was delayed. After opening, the vaginas were found to be variably opened and closed. A significant reduction in P_4 (to less than 1.0 ng/ml) and cessation of P_4 cyclical variation was observed. Plasma E_2 remained high during the whole experimental period. This study shows that cessation of the oestrous cycle by the deslorelin implant might be useful in preventing pregnancy in guinea pigs.

Keywords: guinea pig; deslorelin; progesterone; oestradiol; oestrous cycle

List of abbreviations

E_2 = oestradiol, FSH = follicle-stimulating hormone, GnRH = gonadotropin-releasing hormone, gpGnRH = guinea pig gonadotropin-releasing hormone, LH = luteinising hormone, P_4 = progesterone

Guinea pigs (*Cavia aperea f. porcellus*) are poly-oestrous animals, and sows ovulate spontaneously (Sisk 1976). Guinea pigs have a mean oestrous cycle length of 17.5 ± 2.1 days (range 15–21 days) which is composed of dioestrus, when the female is sexually inactive and the vagina is closed by an epithelial membrane, followed by proestrus and oestrus.

The sow is sexually receptive during proestrus and oestrus and the transition to oestrus is preceded by the opening of the vagina due to dissolution of the vaginal membrane (Stockard and Papanicolau 1917).

Previously, it has been shown that there is a significant elevation of plasma progesterone (P_4) coincident with the luteal phase (dioestrus) in guinea pigs (Feder et al. 1968; Challis et al. 1971; Blatchley

et al. 1976; Garis and Foreman 1984), whereas fluctuations of plasma oestradiol (E_2) levels occur throughout the oestrous cycle (Chalis et al. 1971; Croix and Franchimont 1975; Garris and Foreman 1984; Westfahl and Vekasy 1988; Hutz et al. 1990).

Thus, in the guinea pig, the period of sexual behaviour and receptivity can be indirectly deduced from the duration of dioestrus (when high P_4 levels, low sexual activity and vaginal membranes are present) and oestrus (low P_4 levels, sows are sexually active and vaginal membranes are absent).

Deslorelin acetate is a long-acting synthetic GnRH agonist used for the control of sexual behaviour in various animal species (McRae et al. 1985; Munson et al. 2001; Junaidi et al. 2003; Schoemaker et al. 2008; Romagnoli et al. 2009; Fontaine et al.

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2011; Novotny et al. 2012). Subcutaneous implants provide a long-term, continuous release of small amounts of GnRH. After implantation of deslorelin there is an initial, transient increase in the release of follicle stimulating hormone (FSH) and luteinising hormone (LH), termed 'flare-up'. Depending on at what stage in the oestrous cycle implantation is carried out, the 'flare-up' can be sufficient to induce oestrus and ovulation (Gobello 2007). Continuous, long-term release of deslorelin results in the down regulation of GnRH receptors in the pituitary. This effect is manifested as insensitivity to endogenous GnRH, a consequent failure to release FSH and LH and ultimately, to a failure to induce sexual behaviour. This effect of deslorelin implantation has been harnessed to suppress sexual behaviour and to prevent pregnancy in dogs, cats and ferrets (Munson et al. 2001; Junaidi et al. 2003; Schoemaker et al. 2008; Romagnoli et al. 2009). However, there is insufficient knowledge of the effect of deslorelin in guinea pigs to allow its safe use in practice.

The aim of this study was to determine the effects of deslorelin acetate implants on the oestrous cycle of intact female guinea pigs. Assessment was made before and after deslorelin implantation by monitoring parameters that differentiate stages of the oestrus cycle: the length of oestrus (signalled by disappearance of the vaginal membrane) and changes in plasma hormone E_2 and P_4 levels.

MATERIAL AND METHODS

Animals. Fifteen intact female guinea pigs (*Cavia aperea f. porcellus*) were obtained from an accredited laboratory breeding facility (Velaz, Prague, Czech Republic). They were aged from 3.5–4 months old and weighed 320–400 g. In addition to a standard clinical examination, haematological and plasma biochemistry analyses were performed. No abnormalities were found and the animals were deemed healthy (Quesenberry et al. 2012). The guinea pigs were kept under controlled conditions, i.e.: (1) twelve hours of daylight followed by twelve hours of dark; (2) environmental temperatures between 20–23 °C; and (3) air humidity 41–51%. All animals were fed with a commercial pellet chow (Biokron, Blucina, Czech Republic) and hay. The animals were housed and handled in accordance with the Branch Commission for Animal Welfare of the Ministry of Agriculture of Czech Republic

(accreditation number 50/2010). Guinea pigs were observed daily for signs of sexual behaviour.

Vaginal membrane opening was detected by daily visual inspection of the vagina. Day zero was determined as the day when the guinea pig vagina opened completely (absence of vaginal membrane). End of oestrus was determined by reformation of the vaginal membrane. The subcutaneous implants of deslorelin acetate (Suprelorin 4.7 mg, Virbac, France) were administered at the beginning of the third oestrous cycle, when the vagina was closed.

Hormone assays. Blood samples were collected for plasma E_2 and P_4 determination from day zero and at three-days intervals during the two oestrus cycles and two subsequent cycles after the deslorelin implant insertion, i.e. a total of 66 days. Additional samples were then collected at monthly intervals for up to twelve months after the experiment began. In total, 480 blood samples were collected.

Blood for plasma E_2 and P_4 determination was drawn from the *vena cava cranialis* under general anaesthesia with isoflurane and collected into heparin anticoagulant agent (Jekl et al. 2005). Fresh blood was immediately separated by centrifugation at 5000 g for 8 min.

Plasma E_2 and P_4 concentrations were determined using a chemiluminescent immunoassay method (Immulite 1000, Siemens, USA). The feasibility of using commercial kits prepared for human plasma E_2 and P_4 determination in guinea pigs has previously been validated by others (Rodriguez et al. 2003a; Rodriguez et al. 2003b). The maximal inter-assay coefficient of variation for P_4 was 9.8% and for E_2 it was 7.5%.

Data analyses. Descriptive statistics were expressed as mean values, standard deviations (SD) and range. Statistical analysis was performed with MS-Excel® (Microsoft Corp., Inc., USA) and MedCalc Version 13 (MedCalc Software, Ostend, Belgium). Based on testing for normality (Kolmogorov-Smirnov test), parametric Repeated Measures ANOVA with Bonferroni correction was used for the comparison of P_4 concentrations within the oestrous cycle. Differences in P_4 concentrations before and after deslorelin administration in particular animals were compared with paired two-sample *t*-tests. The same principles were applied to E_2 concentrations. Differences with a value of $P < 0.05$ were considered statistically significant.

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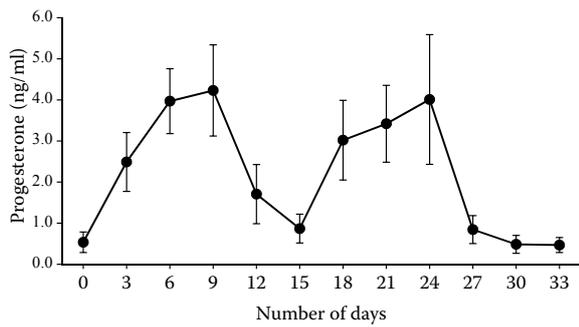


Figure 1. Plasma progesterone (P_4) levels (mean \pm SD ng/ml) in 15 female guinea pigs during two physiological oestrous cycles; Day 0 = first day of the first oestrous cycle

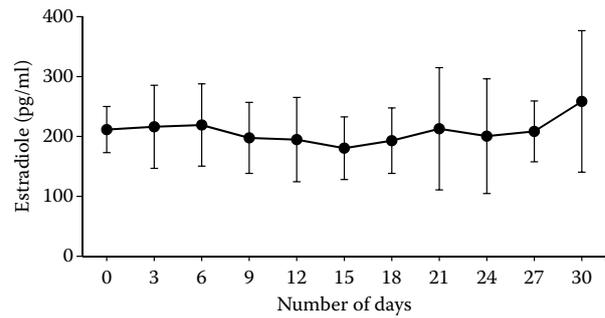


Figure 2. Plasma oestradiol (E_2) levels (mean \pm SD pg/ml) in 15 female guinea pigs during two physiological oestrous cycles; Day 0 = first day of the first oestrous cycle

RESULTS

Two initial oestrous cycles before deslorelin acetate implant administration

The mean length of oestrous cycle was 17.1 ± 2.07 days (range 14–21 days). The proestrus was characterised by increased activity of females, restlessness, swaying motion of the hindquarters and guttural sounds. Throughout this period, the vaginal membrane disappeared and the vagina remained open for two to five days (3.7 ± 1.50 days). In oestrus, females appeared motionless and relaxed when manipulated. Plasma P_4 concentrations showed regular cyclic fluctuations, with the highest individual peak of P_4 of 7.0 ng/ml and lowest of 0.2 ng/ml (day zero of the first oestrous cycle and Day 32 and 33 of the beginning of the third oestrous cycle) (Figure 1). Plasma P_4 concentrations between day three and nine were significantly higher ($P < 0.01$) than those recorded on other days of the oes-

trous cycle. Plasma E_2 concentrations did not show any significant cyclic fluctuations (Figure 2) during the oestrous cycle, with values ranging from 19.9 pg/ml to 119.3 pg/ml, regardless of the phase of oestrous cycle. The mean plasma E_2 concentration was 55.3 ± 18.03 pg/ml.

The effect of deslorelin acetate implant administration

Deslorelin implants were not associated with a complete attenuation of signs of sexual behaviour throughout the experimental period. The duration that the vagina remained open was increased and was found to be more variable after deslorelin implantation (6.7 ± 2.81 ; range five to 12 days) compared to the two prior untreated cycles (3.7 ± 1.50 ; range two to five days). Vaginas were repeatedly open and closed in an irregular pattern. The timing of vaginal opening was some-

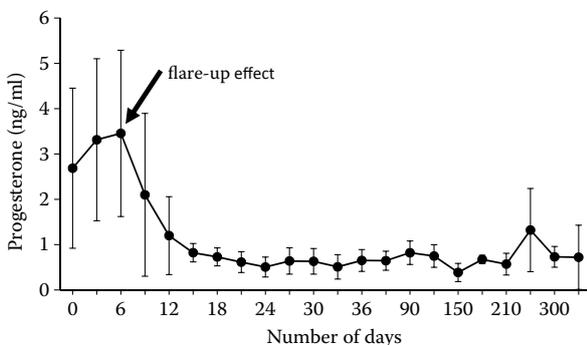


Figure 3. The effect of deslorelin acetate (Suprelorin 4.7 mg) on plasma progesterone (P_4) levels (mean \pm SD ng/ml) in 15 female guinea pigs; Day 0 = deslorelin implant administration

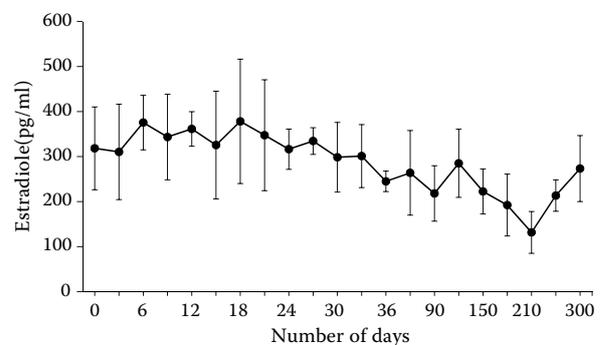


Figure 4. The effect of deslorelin acetate (Suprelorin 4.7 mg) on plasma oestradiol (E_2) levels (mean \pm SD pg/ml) in 15 female guinea pigs; Day 0 = deslorelin implant administration

times longer than the period of vaginal closure. Plasma P_4 concentrations reached their maximum levels six days after implant administration (3.5 ± 1.83 ng/ml). This initial increase (Figure 3, arrow) was followed by a drop to < 1.0 ng/ml (within 15 days), and subsequently to non-detectable values (< 0.2 ng/ml) for the following twelve months (Figure 3). Plasma P_4 concentrations after the flare-up effect were significantly lower in comparison to the physiological oestrous cycle (Days 3, 6, 9) of the particular animals before deslorelin treatment ($P < 0.01$). No change was noticed in plasma E_2 concentrations (Figure 4). Initial “flare-up” effect (nine days after implant administration, 102.3 ± 16.48 pg/ml) was not followed by decline. Plasma E_2 levels ranged from 19.9 pg/ml to 143.8 pg/ml for the following twelve months (Figure 4).

DISCUSSION

The average length of the oestrus cycle in the guinea pigs in the current study was in close agreement with that found by others, (17.5 ± 2.10 days; range 15 to 21 days) (Sisk 1976). Throughout this period, when sows manifested increased activity, restlessness and guttural sounds, vaginal membranes disappeared and the vagina remained open for two to five days (3.7 ± 1.50 days). Opening of the membrane precedes oestrus, but its timing is too variable to be used in accurately establishing the onset of oestrus (Sisk 1976). The monophasic cyclic fluctuations of P_4 coincident with the ovarian luteal phase (dioestrus, lack of sexual behaviour) found in the current study are in accordance with the data of others (Challis et al. 1971; Garris et al. 1984), whereas the E_2 levels in the current study did not show cyclic fluctuation which is in agreement with the non-cyclic fluctuation reported by Croix and Franchimont (1975). These data run counter to previous findings of Hutz et al. (1990) who describe a biphasic follicular growth associated with biphasic E_2 production.

The present study was designed to assess the effects of the deslorelin implant on signs associated with the oestrous cycle, plasma E_2 and P_4 . The suppression of sexual behaviour and permanent presence of a vaginal membrane due to the deslorelin implant was expected. However, we observed that sows exhibited moderate signs of proestrus

throughout the experimental period. Vaginal membranes were opened for five to 12 days (6.7 ± 2.81 days), which was much longer than in a physiological oestrous cycle. Moreover, vaginas were repeatedly open and closed in an erratic pattern. Dissolution of the vaginal membrane precedes oestrus and occurs during proestrus (Sisk 1976). It is likely that the deslorelin implant did not completely suppress sexual behaviour, thus vaginas remained open in a variable fashion. High concentrations of E_2 could affect this irregular course.

P_4 concentrations decreased from day fifteen in all treated females and remained low throughout the study. Instead of an expected decrease of E_2 concentrations after an initial “flare-up” effect, as described in dogs and cats (Garris et al. 1984), E_2 concentrations remained very high in treated guinea pigs during the twelve months of the study. The exact reason for the high E_2 concentrations remains unclear. A possible explanation is that deslorelin acetate in guinea pigs does not diminish FSH release and therefore E_2 concentrations remain high. FSH tends to be secreted consistently and is more dependent on biosynthesis for its secretion, i.e. FSH is not as dependent as LH on GnRH secretion (Millar et al. 2004). Apart from the mammalian type GnRH (mGnRH), guinea pig also has a specific guinea pig GnRH (gpGnRH) (Grove-Strawser et al. 2002; Fujii et al. 2004). Grove-Strawser et al. (2002) found that gpGnRH is able to stimulate the release of LH; however, mGnRH still dominates with its ability to release LH from the pituitary gland. The effect of gpGnRH on the secretion of FSH has not yet been described and its role is still unclear.

CONCLUSION

Deslorelin implantation was associated with altered signs of oestrus. Our data showed a marked effect on P_4 levels. Cessation of P_4 cyclical variation might be useful to prevent pregnancy in guinea pigs. However, intermittent and prolonged vaginal opening associated with deslorelin, which was observed in animals in this study, could potentially predispose them to vaginal infections. At the moment, more studies are necessary to evaluate the impact of treatment with deslorelin acetate on female guinea pigs, especially its effect on E_2 levels.

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