Prolactin, Androstenedione and IGF1 Serum Concentrations During Induced Follicular Growth by eCG Administration in the Bitch

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Contents

The oestrus cycle in the domestic bitch, a monoestrous species, differs considerably from that of other veterinary domestic animals species. In the bitch the combined use of eCG and hCG is effective to induce oestrus predictably and safely (Stornelli et al., Theriogenology, 78, 2012 and 1056). Although several studies were done to describe the hormonal changes during the canine oestrus cycle, to our knowledge none was done to describe the hormonal changes during induced follicular growth after the administration of eCG. The aim of this work was to study prolactin (PRL), insulin-like growth factor (IGF1) and androstenedione (ANDR) serum concentrations during follicular growth induced by a single dose of eCG administered to late anoestrous bitches. PRL and ANDR concentrations were lower before than after eCG TRT (before eCG vs pro-oestrus, oestrus and dioestrus; 4.3 ± 1.8 ng/ml vs 6.5 ± 1.6 ng/ml, $p < 0.05$; 0.08 ± 0.2 ng/ml vs 0.42 ± 0.16 ng/ml, $p < 0.05$). Conversely, IGF1 concentrations were similar before and after eCG TRT (286.0 ng/ml ±32.2, $p > 0.53$). Additionally, PRL concentrations were similar before oestrus compared to during oestrus and dioestrus (6.9 ± 1.7 ng/ml, $p > 0.19$). Furthermore, IGF1 concentrations were higher before and during oestrus compared to first day of dioestrus (286.1 ± 29.8 vs 200.4 ± 29.2 ng/ml, $p < 0.01$). On the contrary, ANDR concentrations were lower before and during oestrus compared to first day of diestrum (0.35 ± 0.17 ng/ml and 0.38 ± 0.15 vs 0.68 ± 0.17 ng/ml, $p < 0.05$). These results show that treatment with a single injection of 50 IU/kg of eCG in late anoestrous bitches successfully induced changes in follicular growth which were paralleled with changes in PRL, IGF1 and ANDR serum concentration similar to those occurring during a normally occurring oestrous cycle. In addition, our results suggest that IGF1 in the bitch could play an important role in ovarian folliculogenesis.

Introduction

The oestrus cycle in the domestic bitch, a monoestrous species, differs considerably from of other domestic animals species. In the bitch, the oestrus cycle is considerably longer than that of most other domestic species. The follicular phase is prolonged, and spontaneous ovulations are followed by a prolonged luteal phase in the non pregnant bitch and by a non-seasonal anoestrus of variable duration (Concannon 1993, 2009). No intrinsic luteolytic mechanism to terminate the corpus luteum in non-pregnant bitches is known; and during luteal phase, serum progesterone is elevated for approximately the duration of pregnancy (Concannon 2009, 2011). The mechanism controlling termination of anoestrus and the start of a new follicular phase in the bitch are poorly understood. Increased basal concentrations and pulsatility of LH have been observed before pro-oestrus, and increase in circulating follicle-stimulating hormone (FSH) concentrations appears to be a critical event in the initiation of ovarian folliculogenesis (Spattini et al. 2007). The follicular phase continues until ovulation. England et al. (2009) suggested follicle recruitment commencing between 100 and 60 days before the pre-ovulatory LH surge. These observations are chronologically similar to the increase in plasma FSH and estrogens (Jeffcoate 1993; Kooistra et al. 1999). The data reported by England et al. (2009) about the follicular diameter in late anoestrus and pro-oestrus suggest a movement from the poll of small follicles to large follicles, presumably reflecting the dynamics of the population of follicles. Unlike most other species, in dogs the duration of the pre-ovulatory LH surge is relatively long (Concannon 1993; Onclin et al. 2002). Follicle-stimulating hormone pulses occur concomitantly with LH pulses in all stages of the oestrus cycle and in anoestrus (Kooistra et al. 1999). The pre-ovulatory LH surge is also associated with a surge in FSH secretion (Olson et al. 1982; Concannon 1993; de Gier et al. 2006). Progesterone is elevated from the LH surge until 18 h before parturition in pregnancy or until 55–75 days in non-pregnant cycles. In each case, progesterone typically peaks at 15–85 ng/ml at 15–25 days and declines slowly thereafter (Concannon 2009).

It is described that there is a large increase in androgen production, presumably from theca cells and serving as a precursor for the enhanced estradiol secretion, and in excess of that required, as it appears as large increases in peripheral testosterone and androstenedione that parallel estradiol concentration (Concannon and Castracane 1985; Concannon 2009). Peripheral progesterone rapidly increases during the pre-ovulatory LH surge, concomitantly with the pre-ovulatory luteinization of follicles (Concannon 2009, 2011).

The control of the PRL secretion involves a complex interrelationship between neurohormones and neurotransmitters in the hypothalamus and plasma levels of ovarian steroids. Prolactin is tonically inhibited by hypothalamic dopamine, the primary-inhibiting factor; and can be stimulated by a number of neurohormones

acting as prolactin-releasing factors. Among neuro-transmitters, serotonin has been demonstrated to stimulate prolactin release (Szawka et al. 2007).

In the bitch, in addition to hypothalamic inhibitory stimulatory signals, gonadal steroids modulate the pituitary secretion of PRL. Conversely, PRL may influence the secretions of gonadotropins. A pre-ovulatory prolactin surge has been reported in other species, but its occurrence and relevance in bitches remain to be elucidated (de Gier et al. 2006).

Additionally, insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) in ovarian folliculogenesis have been extensively studied in several mammalian species (De la Sota et al. 1996). In all these species, IGFs and several low-molecular-weight IGFBPs (in particular IGFBP-2 and IGFBP-4) are considered, respectively, as stimulators and inhibitors of follicular growth and maturation (De la Sota et al. 1996; Mazerbourg et al. 2003; Lenz Souza et al. 2007; Silva et al. 2009). There is evidence in several studies for the importance of IGFs in follicular growth in animals (Eden et al. 1988; Oliver et al. 1989; Richards et al. 1991; De la Sota et al. 1996; Huang et al. 1997; Bachelot et al. 2002; Mazerbourg et al. 2003; Silva et al. 2009).

In the bitch, combined use of eCG and hCG is effective to induce oestrus predictably and safely (England et al. 2009; Stornelli et al. 2012). A protocol using 50 IU/kg of eCG combined 7 days later with 500 IU of hCG induced normal and fertile oestrus at 164 days post-oestrus with an 80% pregnancy rate (Stornelli et al. 2012). Although several studies were done to describe the hormonal changes during the canine estrous cycle, to our knowledge none was done to describe the hormonal changes during induced follicular growth after the administration of eCG.

The aim of this work was to study prolactin (PRL), insulin-like growth factor (IGF1) and androstenedione (ANDR) serum concentrations during induced follicular growth by a single dose of eCG administered to late anoestrous bitches.

Materials and Method

Experimental Design

Six healthy, intact mixed breed bitches aged between 2 and 7 years old and weighing between 10 and 27 kg were used in this study. All animals were individually housed in pens at the University Veterinary Teaching Hospital kennel. The day of anoestrus was determined based on the history provided by the owner and confirmed in each bitch based on serum progesterone (<1 ng/ml) and sexual behaviour (SB), clinical signs of oestrus (CSE), vaginal cytology (VC) score. In all bitches, anoestrus was characterized by the preceding mean interoestrus interval (200.4 ± 6 days). The first day of the treatment (TRT), each bitch received a single dose of eCG (50 IU/kg, im). Sexual behaviour, CSE, and VC samples were obtained before eCG administration

and every other day until the first day of cytological dioestrus. Briefly, each study animal was observed for SB and CSE and scored from 1 to 3 (Table 1; Stornelli et al. 2012). Swabs were introduced 6–10 cm into the vagina, and quickly and gently rotated against the floor and lateral walls of the vagina. Smears were air-dried and stained with methylene blue. Stained slides were examined at $100\times$ and $400\times$ magnification to quantify parabasal, intermediate and superficial cells. Stage of the oestrous cycle was determined according to the percentage and type of cells present (Feldman and Nelson 1987). According to the percentage and type of cells present, samples were scored from 1 to 5 (Table 2; Stornelli et al. 2012).

Blood samples were taken before eCG administration and twice a day until first day of cytological dioestrus. All blood samples were centrifuged and stored at $-20°C$ until serum hormone concentration were measured. Progesterone and androstenedione were measured by radioimmunoassay (RIA) with a solid-phase radioimmunoassay (RIA) using I125 (Coat-A-Count, androstenedione; Coat-A-Count, Progesterone; Diagnostic Product Corporation, Los Angeles, CA, USA). IGF1 was measure using ethanol extraction (Breier y col 1991; Diaz-Torga y col 2002) and PRL was measured by ELISA (canine prolactin ELISA MKVCP1; Demeditec Diagnostic Gmbh Kiel, Germany).The intra-assay CVs for high-pool and low-pool P_4 (5.6 and 1.0 ng/ml) were 3.4% and 6.1% respectively.

Statistical analysis

The SB + CSE and VC scores were analysed by the GENMOD procedure and the hormonal concentrations

Table 1. Sexual behaviour and clinical signs of oestrous score[a]

Score	Sexual behaviour	Clinical signs
1	No attraction to the male	No external signs of oestrus[b]
2	Attraction to the male but no receptivity to mating	Poor external signs of oestrus[b]
3	Attraction to the male and receptivity to mating	Significant external signs of oestrus[b]

[a]Adapted from Feldman and Nelson (1987) and Stornelli et al. (2012).
[b]Serosanguineous discharge, swollen and turgid vulva.

Table 2. Vaginal cytology score[a]

	Vaginal cytology				
Score	Superficial cells	Intermediate cells	Parabasal cells	Erythrocytes	Neutrophils
1	5–10%	80–90%	5–10%	–	–
2	20–30%	65–70%	0–5%	–	–
3	60%	40%	–	–	–
4	90–95%	5–10%	0–5%	Few	–
5	90–95%	5–10%	0%	Many	+

[a]Adapted from Feldman and Nelson (1987).

by the MIXED procedures of SAS. Data are presented as LSM ± SEM.

Significance was defined as $p < 0.05$ (SAS 1989).

Results

At the beginning of the study before eCG administration, anoestrus was confirmed in all animals by SB + CSE and VC mean scores (1; $p < 0.01$; 1, $p < 0.01$; respectively) and mean serum concentrations of P_4 (0.23 ± 0.08 ng/ml). All bitches responded to treatment by coming into clinical oestrus (SB + CSE, 1.9, $p < 0.01$; VC, 1.9, $p < 0.01$) within 2–7 days after eCG administration (3.3 ± 0.94 days). Both SB + CSE and VC were similar to those observed in spontaneous cycles. However, based upon P_4 concentrations on the first day of cytological dioestrus (SB + CSE, 1.0, $p < 0.01$; VC, 3.0, $p < 0.01$), three bitches failed to have ovulation (FAIL) and three bitches had ovulation (OVUL; 2.80 vs 85.33 ± 6.18 ng/ml; $p < 0.01$). Conversely, P_4 concentrations at the time of eCG administration were similar among all bitches (0.23 ± 0.08 ng/ml; $p > 0.37$). The interval from eCG to oestrus (ITE) was similar in all bitches (3.3 ± 0.94 days). However, the interval from oestrus to first day of cytological dioestrous (IED), and the interval from eCG administration to first day of cytological dioestrus was shorter in the bitches in the FAIL group compared to OVUL group (5.00 vs 10.00 ± 1.31 days, $p < 0.05$; 7.66 vs 14.66 ± 0.78 days, $p < 0.01$). PRL and ANDR concentrations were lower before than after eCG TRT (before eCG vs pro-oestrus, oestrus and dioestrus; 4.3 ± 1.8 vs 6.5 ± 1.6 ng/ml, $p < 0.05$; 0.08 ± 0.2 vs 0.42 ± 0.16 ng/ml, $p < 0.05$). Conversely, IGF1 concentrations were similar before and after eCG TRT (286.0 ± 32.2 ng/ml, $p > 0.53$). Additionally, PRL concentrations were similar before oestrus compared to during oestrus and dioestrus (6.9 ± 1.7 ng/ml, $p > 0.19$). Furthermore, IGF1 concentrations were higher before and during oestrus compared to first day of dioestrus (286.1 ± 29.8 ng/ml vs 200.4 ± 29.2 ng/ml, $p < 0.01$). On the contrary, ANDR concentrations were lower before and during oestrus compared to first day of dioestrus (0.35 ± 0.17 ng/ml and 0.38 ± 0.15 ng/ml vs 0.68 ± 0.17 ng/ml, $p < 0.05$).

Discussion

Our results show all bitches responded to treatment by coming into clinical oestrus within 2–7 days after eCG administration and both SB + CSE and VC were similar to those observed in spontaneous cycles. These results are in agreement with previous findings from Stornelli et al. (2012), who observed that 50 UI of eCG induced follicular growth in all animals studied.

Even though in the last three decades, several protocols for pharmacological induction of oestrus in bitches have been studied, same disadvantages can be listed. These protocols include the use of dopamine agonists (bromocriptine and cabergoline), GnRH agonists (lutrelin, buserelin, fertirelin, deslorelin and leuprolide) and exogenous gonadotropins (LH, FSH, hCG, eCG and hMG) (Fontaine et al. 2011; Stornelli et al. 2012; Thun et al. 1977; Wright 1980). Deslorelin is useful to induce oestrus and ovulation in the bitch and is often used in Europe; however, some of these GnRH agonist protocols are expensive or may not be available in all countries. A disadvantage of induction of oestrus using dopamine agonists is that this method may require over 30 days of treatment before the onset of pro-oestrus depending of the stage of anoestrus (Verstegen et al. 1999; Kutzler 2005; Kutzler 2007; Cirit et al. 2007), and that bromocriptine commonly induces vomiting.

Recently, Stornelli et al., showed that a protocol using 50 IU/Kg of eCG combined 7 days later with 500 IU of hCG induced normal and fertile oestrus in the bitch. According to results from the present study, the use of a single dose of 50 IU eCG induces follicular growth but may not be suitable to induce ovulation and increase serum progesterone in all bitches. These results support the fact that the use of hCG is necessary to induce ovulation in the majority of individuals in spontaneous ovulators such us bitches, cows, ewes, mares and sows (Menchaca and Rubianes 2004; De Rensis et al. 2006; Evans et al. 2006). The results of the present study are in agreement with those of Stornelli et al. (2012) and England et al. (2009), who used a protocol combining eCG followed hCG for oestrus induction in the bitch obtaining an increase of progesterone in 100% of treatment bitches (England et al. 2009 and Stornelli et al. 2012). While eCG induces follicular development and in some bitches allows spontaneous ovulation, not all animals will ovulate spontaneously with a single eCG dose. Hence, the use of hCG after eCG treatment will induce ovulation in all bitches.

Although these studies from England et al. and Stornelli et al. show that a combination of eCG and hCG induced normal and fertile oestrus in the bitch, further studies are needed to confirm the fertility and prolificacy results obtained with this protocol.

We found an increase of androstenedione levels after eCG treatment. In this way, our results are in agreement with Concannon and Castracane (1985) who found high androstenedione serum concentration in both pregnant and non-pregnant bitches during follicular and luteal phase. Furthermore, the range of androstenedione blood concentration found in our work in the first day of cytological dioestrus was similar to those reported by Concannon and Castracane (1985) during early dioestrus.

There are several studies on serum prolactin concentration during the oestrous cycle in the bitch. Spattani and Olson reported higher variability among animals in this hormone during oestrus (Olson et al. 1982; Spattini et al. 2007). However, de Gier et al. (2006) showed that prolactin concentration remained constant throughout the oestrus cycle. In agreements with those findings our

results did not show changes in prolactin concentrations during the oestrus cycle.

There is evidence that IGF1 plays a crucial role in ovarian folliculogenesis and female fertility (Eden et al. 1988; Huang et al. 1997; Oliver et al. 1989; De la Sota et al. 1996). The findings of Eden et al. (1988) and Oliver et al. (1989) suggest that IGF1 plays a key role in the responsiveness of the ovary to the FSH action. In the sheep as well as in the rat, pig and human, IGF-1 stimulates both proliferation and differentiation of granulosa cells (Adashi et al. 1992; Giudice 1992). In cattle, IGF-1 stimulates growth of cultured small antral follicles (281–380 mm) and improves oocyte viability (Silva et al. 2009). Furthermore, IGF-1 stimulates proliferation and augments secretion of estradiol in cultured porcine theca cells derived from antral follicles (Silva et al. 2009). In agreement with those studies, in our work, IGF1 serum concentrations were higher during oestrus compared to dioestrus in the bitch. This finding suggests that IGF1 could play an important role in follicular growth in the bitch. In the same way, a positive correlation between plasmatic and intrafollicular levels of IGF-1 had been shown in the woman, mare, cow, ewe and bitch (De la Sota et al. 1996; Mazerbourg et al. 2003; Reynaud et al. 2010). Every change in IGF-1 levels in serum seems to have a direct impact on the level in large antral follicles (Reynaud et al. 2010). Furthermore, IGF-binding proteins (IGFBP3) increase showed a positive correlation between plasmatic and intrafollicular levels (pre-ovulatory follicles; De la Sota et al. 1996; Reynaud et al. 2010). These studies support our results in which IGF-1 serum concentration is higher during follicular growth and oestrus compared to IGF-1 serum concentration in dioestrus. The increasing of IGF-1 in follicular fluid would be responsible of IGF-1 increase in serum.

In conclusion, eCG treatment induced changes in follicular growth paralleled by changes in PRL, ANRD and IGF1, which were similar to those occurring in spontaneous natural oestrus cycles in the bitch. IGF1 could play an important role in ovarian folliculogenesis.

Acknowledgements

Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

All authors contributed equally.

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