

Influence of the dopamine antagonist domperidone on the vernal transition in seasonally anoestrous mares

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The aim of the present study was to determine the effect of the prolonged administration of the dopamine antagonist domperidone on follicular development, ovulation and endocrine profiles in anoestrous mares. Anoestrous mares ($n = 16$) were maintained under natural photoperiod and ambient temperature. Eight of the mares were treated with domperidone each day from 15 January until the first ovulation of the year. The mean number and size of follicles ≥ 20 mm in diameter were significantly greater in domperidone-treated mares than in control mares by day 14 of treatment. The day of first ovulation was significantly earlier in domperidone-treated mares than in control mares (mean \pm SEM: 51 ± 8.2 and 129 ± 13.6 days, respectively; $P < 0.01$). The eight domperidone-treated mares all ovulated during treatment and the mean interval from the start of treatment to the day of first ovulation was 27 days (range 15-55 days). Six of the eight domperidone-treated mares underwent normal cycles after first ovulation, whereas there was a prolonged interval (mean = 67 days) before the second ovulation in the other two mares. Domperidone administration resulted in significantly higher plasma prolactin concentrations measured at 0,4,8,12, and 24 h on day 7 of treatment compared with untreated controls (25.5 ± 15.8 versus 2.5 ± 3.0 ng ml⁻¹). The concentrations of LH and oestrogen conjugates were significantly higher in domperidone-treated mares compared with control mares by day 28 of treatment. There were no differences in FSH concentrations in domperidone-treated and control mares. The significant increases in follicular development and concentrations of oestrogen conjugates that were observed to occur without concurrent increases in FSH concentration indicate that either domperidone or prolactin may have a direct effect on the ovary.

Introduction

Studies of the hormonal events associated with the resumption of reproductive activity during the vernal transition in mares have focused primarily on the secretion of hypothalamic GnRH and the gonadotrophins FSH and LH (Robinson *et al.*, 1995). The vernal transition is initiated by increased synthesis and secretion of GnRH, which stimulates FSH release. GnRH also stimulates synthesis of LH, but the ultimate release of LH, which leads to the first ovulation of the year, also requires a contribution from the ovary. The stimulus for LH release is the development of steroidogenically competent

follicles, which results in significant increases in follicular fluid and peripheral oestrogen concentrations (Sharp *et al.*, 1991; Cleaver and Sharp, 1995). Pituitary LH content and peripheral LH concentrations increase coincidentally with oestradiol concentrations, culminating in the first ovulation of the year and initiation of the ovulatory season.

A third hormone, prolactin, has also been demonstrated to have a gonadotrophic function in mammals (Doppler, 1994; Wise and Maurer, 1994; Lebedeva *et al.*, 1998). The role of prolactin in regulating reproductive activity in mares has not been investigated fully and there are conflicting reports. Besognet *et al.* (1995) monitored prolactin concentrations in mares during the vernal transition in relation to the time of onset of first ovulation. Besognet *et al.* (1995) concluded that the onset of the reproductive season, in relation to the first ovulation, might be independent of increasing prolactin concentrations. In contrast, Nequin *et al.* (1993) observed that artificially increasing endogenous prolactin concentrations during anoestrus, either by dopamine receptor blockade or by administering exogenous prolactin, resulted in rapid follicular growth. This result indicates that prolactin may have a direct stimulatory effect, possibly on the ovary. Prolactin receptors have been identified in the ovaries of several mammalian species and have been implicated in regulatory activity (Besognet *et al.*, 1997). Lebedeva *et al.* (1998) demonstrated that there is a direct relationship between follicular fluid prolactin concentrations and follicular atresia in cattle. Follicles that had the morphological and microscopic characteristics of 'non-atretic' follicles (fine, prevalent vasculature with a low percentage (< 20%) of pycnotic nuclei) had significantly higher follicular fluid prolactin concentrations than did follicles characterized as 'atretic' (poor vasculature and a high percentage (> 40%) of pycnotic nuclei).

Besognet *et al.* (1997) demonstrated that dopamine has a major role in regulating prolactin secretion in most species, including mares. The influence of dopamine on reproductive function in seasonally anoestrous mares is poorly understood. Dopamine may exert tonic inhibition on reproductive activity during the anovulatory season (Besognet *et al.*, 1997). The finding that dopamine concentrations are highest in the cerebrospinal fluid of mares during the anovulatory season and are inversely correlated with LH concentrations supports this hypothesis. There also appears to be a functional relationship between dopamine secretion and ovarian activity. Seasonal variations in LH secretion are observed in ovariectomized mares, although there are no seasonal differences in the dopamine concentrations in the cerebrospinal fluid of these mares (Melrose *et al.*, 1990). Administration of the dopamine antagonist, sulpiride, to seasonally anoestrous mares resulted in significant transient increases in prolactin concentrations and increased time to first ovulation (Besognet *et al.*, 1997). Sulpiride crosses the blood-brain barrier and, thus, could potentially affect both hypothalamic and pituitary secretion, as well as secretion at the ovary. Domperidone is a specific D₂ dopamine receptor antagonist that does not cross the blood-brain barrier (Olin *et al.*, 1991). Redmond *et al.* (1997) demonstrated that administration of multiple doses of domperidone induces significant and prolonged (> 24 h) increases in prolactin concentrations.

The aim of the present study was to test the hypothesis that artificial maintenance of significantly increased prolactin concentrations by prolonged treatment with domperidone would affect hormone secretion and reproductive function in anoestrous mares.

Materials and Methods

Animals and experimental design

Non-pregnant mares ($n = 16$) of mixed breeding, aged 3-15 years, mass 400-600 kg, were used. All mares were reproductively sound and underwent normal oestrous cycles in the year before the present study. The mares were all maintained at the same location (Tuskegee, AL, USA; latitude 32.43 north°) for at least 1 year before the present study began. Mares were maintained under natural photoperiod in dry lots and fed a balanced ration of grass hay, grain and mineral supplements, with free access to water. When the study began, all the mares were confirmed to be undergoing seasonal anoestrus, based on measurements of plasma progesterone concentrations ($< 1.0 \text{ ng ml}^{-1}$) in three samples taken once a week in December and January.

Mares were divided randomly into two groups. From 14 January (day of the year = 14) mares in the treatment group received an oral dose (1.1 mg kg^{-1}) of domperidone in a molasses base (Equitox, Clemson, SC) once each day until the first ovulation of the year, for a maximum of 55 days. Mares in the control group received the molasses base orally each day until the first ovulation of the year. The reproductive activity of each mare was monitored by teasing, transrectal palpitation and ultrasonography (Alokoa 500, 5 Mhz linear transducer; Corometrics Medical Systems, Wallingford) three times each week on Monday, Wednesday and Friday. The endpoints were the number and size of follicles $\geq 20 \text{ mm}$ in diameter, ovulation and presence of corpora lutea. The day of ovulation was considered to be the day of loss of follicles $\geq 30 \text{ mm}$ in diameter between two examinations and the identification of corpora lutea by ultrasonography. Ovulation was confirmed by measurement of an increase in plasma progesterone concentration to $\geq 1.0 \text{ ng ml}^{-1}$. Blood samples were collected each day between 07:00 h and 08:00 h into evacuated heparinized tubes before the reproductive activity examination. Plasma was separated within 3 h of collection by centrifugation at 1500 g for 15 min and frozen at -20°C until assayed. Ambient outside temperature (high and low) was recorded at the National Weather Service, Montgomery, AL.

Treatment

Domperidone is a specific dopamine D_2 receptor antagonist. Unlike other neuroleptics with anti-dopaminergic action investigated in horses, namely perphenazine and sulpiride (Nequin *et al.*, 1993; Besognet *et al.*, 1997), domperidone does not cross the blood-brain barrier (Olin *et al.*, 1991).

Domperidone (1.1 mg kg^{-1}), suspended in molasses, was administered orally once each day between 08:00 h and 10:00 h. The dose of domperidone administered to the mares was determined from a study that used domperidone for treating fescue toxicoses in pregnant mares (Redmond *et al.*, 1997). Blood was collected at times 0, 4, 8, 12 and 24 h in the 24 h after domperidone administration to demonstrate a pharmacological effect of the domperidone. Additionally, samples were collected five times on each of days 7, 14 and 28 from domperidone-treated and control mares. Harvested blood was collected into heparinized tubes and stored on ice until centrifugation. Samples were centrifuged at 1500 g for 15 min, and the plasma was collected and stored frozen at -20°C until assayed

for hormone content. The concentrations of prolactin, oestrogen conjugates, FSH and LH were measured in all samples.

Hormone analyses

Prolactin assay. Prolactin concentrations were determined using a homologous double-antibody radioimmunoassay (RIA) (Besognet *et al.*, 1995) containing rat anti-equine prolactin antibody (Harbor-UCLA Medical Center, Torrance). Purified equine prolactin (AFP-7730B; Harbor-UCLA Medical Center) was used as a standard and for iodination. The limit of sensitivity of the assay was 0.1 ng ml⁻¹. All samples were run in a single assay. The mean intra-assay coefficient of variation was 9.0%.

LH assay. Plasma LH concentrations were determined by heterologous double-antibody RIA (Roser and Hughes, 1991). Highly purified equine LH (E263B; University of California, Davis) was used for the standards and iodination, and the first antibody was a mouse anti-bovine LH- β monoclonal antibody (518B7; University of California). The limit sensitivity of the assay was 0.5 ng ml⁻¹. The mean intra- and inter-assay coefficients of variation were 11.0 and 9.0%, respectively.

FSH assay. Plasma FSH concentrations were determined by heterologous double-antibody RIA (Roser and Hughes, 1991). Highly purified equine FSH (E265B; University of California) was used for standards and iodination, and the first antibody was a rabbit anti-human FSH (anti-human FSH-6 antisera AFP-005; National Hormone and Pituitary Program, Rockville). The limit of sensitivity of the assay was 1.0 ng ml⁻¹. The mean intra- and inter-assay coefficients of variation were 8.0 and 9.0%, respectively.

Oestrogen conjugate assays. The concentrations of plasma oestrogen conjugates were determined in unextracted plasma (Daels *et al.*, 1991). The limit of sensitivity of the assay was 0.2 ng ml⁻¹. The mean intra- and inter-assay coefficients of variation were 8.7 and 12.0%, respectively.

Progesterone assay. Progesterone concentrations were determined in unextracted plasma samples using a solid phase I¹²⁵ RIA kit (Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles). The sensitivity of the assay was 0.1 ng ml⁻¹ and the intra-assay coefficient of variation was 6.5%. All samples were run in a single assay.

Statistical analyses

Data on time to ovulation were compared between groups by Student's *t* test. Mean gonadotrophin and hormone secretion was evaluated by calculating the areas under the curves of plasma concentrations plotted against time for each individual mare. Comparisons of hormone secretion and follicular development between treatment groups were made by analysis of variance evaluating the main effects of treatment on follicle size, number, LH, FSH, prolactin and oestrogen conjugate concentrations. Pairwise comparisons of treatment means were made by the Least Significant Difference (LSD)

method. Differences were considered significant at $P < 0.05$. Data are reported as mean \pm SEM.

Results

Follicular development and time to first ovulation

The mean numbers and sizes of follicles were greater in domperidone-treated mares than control mares by day 14 (mean numbers: $n = 4.4$ and $n = 2.1$; mean sizes: 27.2 ± 5.2 and 15.1 ± 3.8 mm, respectively; $P < 0.01$) (Fig. 1). The mean day of ovulation was significantly earlier for domperidone-treated mares than control mares (51 ± 8.2 ($n = 8$) and 129 ± 13.6 ($n = 8$), respectively; $P < 0.01$). All eight domperidone-treated mares ovulated during treatment and the mean interval from the start of treatment to ovulation was 27 days (range 15-55 days). Six of the eight domperidone-treated mares continued to undergo normal cycles after the first ovulation, whereas the other two mares had a prolonged interval (mean = 67 days) until the second ovulation.

Plasma hormone concentrations

Prolactin. Domperidone administration resulted in significantly higher plasma prolactin concentrations in mares at 4, 8, 12 and 24 h on day 1 of treatment compared with untreated control mares (Fig. 2). On day 7 of treatment, prolactin concentrations were significantly higher 0, 4, 8, 12, and 24 h after domperidone administration compared with control mares (Fig. 3), and remained comparably higher at all subsequent times (Fig. 4).

LH, FSH and oestrogen conjugates. FSH, LH and oestrogen conjugate concentrations did not significantly increase compared with time 0 at 4, 8, 12, and 24 h on days 1, 7, 14 and 28 and so the mean concentrations on each sampling day were determined. Mean FSH concentrations did not differ between control and domperidone-treated mares in any sampling period (3.2 ± 1.1 and 3.8 ± 1.2 ng ml⁻¹, week 1; 4.8 ± 2.2 and 5.2 ± 2.5 ng ml⁻¹ week 2; 5.5 ± 2.0 and 4.5 ± 1 ng ml⁻¹, week 4; respectively) (Table 1). Mean concentrations of LH and oestrogen conjugates were significantly greater in domperidone-treated mares than untreated mares by day 28 of treatment (LH: 2.3 ± 2.2 and 7.9 ± 7.2 ng ml⁻¹, respectively; $P < 0.05$; oestrogen conjugates: 0.5 ± 0.8 and 1.5 ± 1.1 ng ml⁻¹, respectively; $P < 0.05$; Table 1).

Progesterone. Plasma progesterone concentrations remained high (≥ 2.0 ng ml⁻¹) for 14 days after ovulation in the six domperidone-treated mares that continued to cycle, indicating normal luteal function. Progesterone concentrations in the two mares that did not continue to cycle were < 1.0 ng ml⁻¹ by day 7 after ovulation.

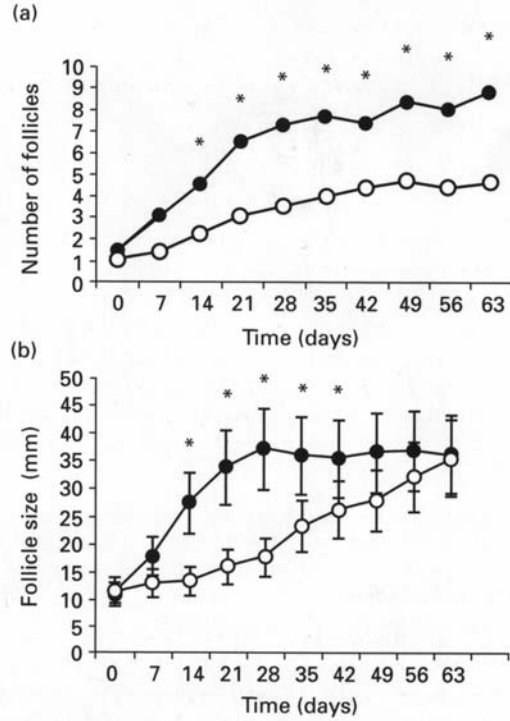


Fig. 1. (a) Number of follicles ≥ 20 mm in diameter and (b) size of follicles in untreated mares ($n = 8$; ○) and domperidone-treated mares ($n = 8$; ●) during domperidone treatment from day 0 to day 63. *Values for domperidone-treated and control mares are significantly different ($P < 0.05$) at this time.

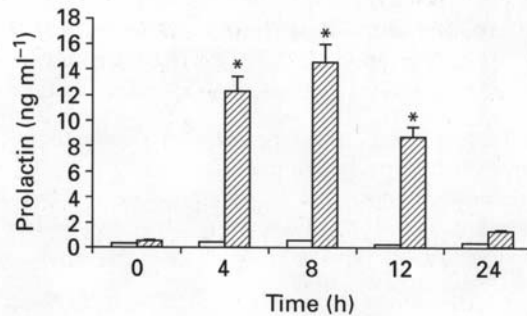


Fig. 2. Plasma prolactin concentrations on day 1 of treatment at times (t) 0, 4, 8, 12 and 24 h in untreated mares ($n = 8$; □) and domperidone-treated mares ($n = 8$; ▨) after domperidone administration at $t = 0$ h. Values are mean \pm SEM. *Values for domperidone-treated and control mares are significantly different ($P < 0.05$) at this time.

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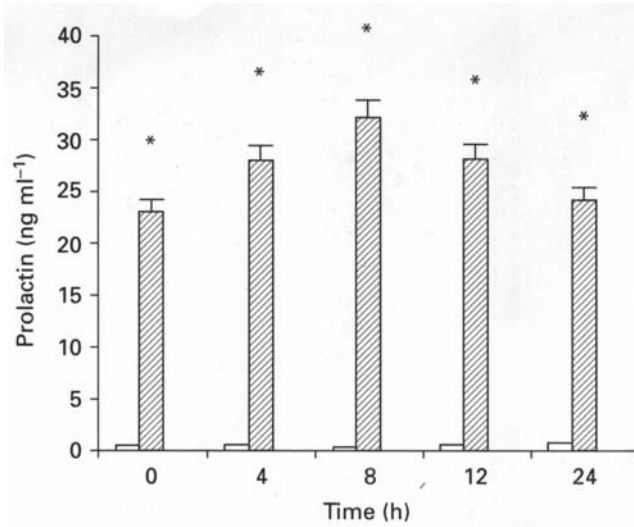


Fig. 3. Plasma prolactin concentrations on day 7 of treatment at times (*t*) 0, 4, 8, 12 and 24 h in untreated mares ($n = 8$; □) and domperidone-treated mares ($n = 8$; ▨) after domperidone administration at $t = 0$ h. Values are mean \pm SEM. *Values for domperidone-treated and control mares are significantly different ($P < 0.05$) at this time.

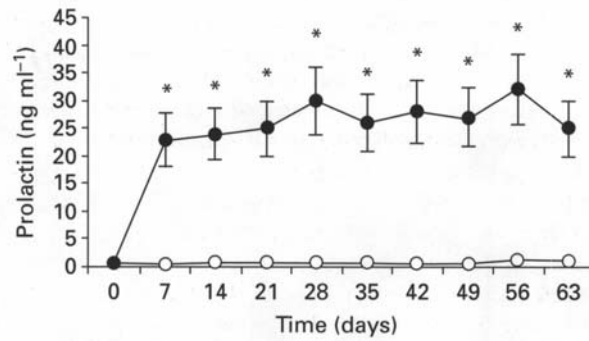


Fig. 4. Plasma prolactin concentrations throughout the entire treatment period in untreated mares ($n = 8$; ○) and domperidone-treated mares ($n = 8$; ●) after domperidone administration at $t = 0$ h. Values are mean \pm SEM. *Values for domperidone-treated and control mares are significantly different ($P < 0.05$) at this time.

Table 1. Plasma FSH, oestrogen conjugate and LH concentrations in weeks 1, 2 and 4 for control and domperidone-treated mares

Day	Control mares (<i>n</i> = 8)			Domperidone-treated mares (<i>n</i> = 8)		
	FSH (ng ml ⁻¹)	Oestrogen conjugate (ng ml ⁻¹)	LH (ng ml ⁻¹)	FSH (ng ml ⁻¹)	Oestrogen conjugate (ng ml ⁻¹)	LH (ng ml ⁻¹)
1	3.2 ± 1.1	3.2 ± 1.1	0.2 ± 0.2	3.8 ± 1.2	0.3 ± 0.4	1.8 ± 0.7
14	4.8 ± 2.2	0.3 ± 0.4	1.5 ± 1.2	5.2 ± 2.5	0.5 ± 0.2	2.4 ± 1.9
28	5.5 ± 2.0	0.5 ± 0.8 ^a	2.3 ± 2.2 ^c	4.5 ± 1.8	1.5 ± 1.1 ^b	7.9 ± 2.2 ^d

Values are mean ± SEM plasma concentrations collected at 0, 4, 8, 12 and 24 h after administration of domperidone (1.1 mg kg⁻¹; Equitox, Clemson) on days 1, 14 and 28 after the start of treatment.

Separate comparisons were made between treatments in a given row.

^aValues are significantly different between the two treatment groups (*P* < 0.05).

^cValues are significantly different between the two treatment groups (*P* < 0.05).

Discussion

The results of the present study demonstrate that treatment with the dopamine antagonist domperidone stimulates significant increases in prolactin concentrations, which are maintained at supraphysiological concentrations. By day 7 of treatment, prolactin concentrations remained high for 24 h, indicating that domperidone was having a sustained pharmacological effect. Significant increases in the sizes and numbers of follicles were evident by day 14 of treatment. Besognet *et al.* (1995) demonstrated that an increase in prolactin concentrations is not essential for resurgence of ovarian activity, as mares can initiate cyclicity independently of a measurable increase in prolactin concentrations.

However, Nequin *et al.* (1993) proposed that prolactin might have a stimulatory effect on follicular development in mares during the vernal transition. Nequin *et al.* (1993) observed that a single administration of ovine prolactin to anoestrous mares in December stimulated significant increases in follicular growth in the subsequent weeks. Although prolactin receptors have not been identified on the ovaries of mares, prolactin receptors have been identified in several other species and may have regulatory roles. Concentrations of prolactin in follicular fluid from atretic follicles in cows were significantly lower than the concentrations in fluid from follicles with normal gross and histological morphology (Lebedeva *et al.*, 1998). Significant accumulations of Ca²⁺ in intracellular stores in granulosa and thecal cells of follicular walls were observed concurrently with lower follicular prolactin concentrations in atretic follicles (Lebedeva *et al.*, 1998). Addition of prolactin to cultures of follicular wall cells resulted in the release of significant amounts of Ca²⁺ from intracellular stores. These results support the

hypothesis that prolactin may have a regulatory role through mobilization of Ca^{2+} from intracellular stores in cells that are responsive to its mitogenic action (Doppler, 1994).

The normal transition from anoestrus to reproductive cyclicity is associated with an increase in the frequency of pulsatile LH and FSH secretion (Alexander and Irvine, 1991). In the present study, domperidone administration had no pharmacological effects on FSH, LH and oestrogen conjugate secretion. Mean concentrations of FSH did not differ among domperidone-treated and control mares. These results are consistent with studies which demonstrated that FSH concentrations in intact Pony (Turner *et al.*, 1979a) and horse (Thompson *et al.*, 1986) mares did not differ significantly among months or seasons. Although it has been demonstrated that there is an association between FSH concentrations and the degree of follicular activity during the anovulatory season within individual mares (Turner *et al.*, 1979b) the FSH concentrations were essentially the same between domperidone and control mares in the present study.

Mean concentrations of oestrogen conjugates and LH were increased in domperidone-treated mares on day 28 of treatment. The increased oestrogen concentrations that are observed to occur without comparable increases in FSH concentrations indicate that either domperidone or prolactin may have a direct effect on the ovary. Prolactin receptors are present in the ovaries of several mammalian species and have been implicated as having stimulatory roles (Besognet *et al.*, 1997). A direct effect of domperidone via dopaminergic receptors is also a possibility. Support for dopaminergic regulation of seasonal reproductive activity is well described for ewes (Havern *et al.*, 1991) and is further supported by the presence of synapses between dopaminergic neurones and GnRH neurones at the median eminence in sheep (Kulijis and Advis, 1989). The negative feedback of oestradiol in sheep is mediated by dopamine via D_2 dopamine receptors (Havern *et al.*, 1994). The advanced onset of reproductive activity in anoestrous mares treated with the dopamine antagonist sulpiride may be due to central blockade of dopamine D_2 receptors that are associated with inhibition of GnRH secretion. Domperidone does not cross the blood-brain barrier, indicating that its site of action is likely to be distal to the hypothalamus and to involve factors other than stimulation of GnRH release. A direct stimulatory effect of prolactin or domperidone on LH synthesis and secretion is a possibility that requires further investigation.

Sharp (1998) proposed that the first ovulatory LH surge during the vernal transition in mares might be controlled by positive feedback of ovarian oestrogens on LH secretion. The potential role of ovarian steroid feedback is thought to be limited to the end of the vernal transition, as concentrations of oestradiol in the circulation are minimal until late in transition. Other factors, specifically photoperiod, are also likely to be involved, as Cleaver and Sharp (1995) demonstrated that anoestrous mares subjected to ambient daylength and to which oestradiol was administered in December did not undergo an LH response, whereas a combination of stimulatory photoperiod and oestradiol administration led to enhanced LH secretion. The number of GnRH receptors on the pituitary gland are the same in anoestrus and oestrus, and so the positive LH response is apparently due to renewed LH synthesis. Plasma concentrations of LH were increased in the present study by day 28 of domperidone treatment, which was significantly earlier than in the untreated mares maintained under the same ambient photoperiod. These results indicate that synthesis of LH in the pituitary is influenced by dopaminergic mechanisms independent of GnRH control.

The results of the present study demonstrate that administration of the specific D₂ dopamine receptor antagonist, domperidone, early in the vernal transition is associated with prolonged maintenance of high prolactin concentrations and a significantly earlier onset of reproductive activity. In addition, the secretion of oestrogens and LH was increased compared with concentrations typically observed in late transition. Further studies are needed to clarify the roles of prolactin and dopamine on folliculogenesis and steroidogenesis at the level of the ovary, as well as their influence on LH synthesis and secretion during the vernal transition.

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