University of Veterinary Medicine
Department and Clinic of Reproduction

Pseudopregnancy of the bitch in connection to prolactin

by
Anastasia Alexia Papanikolaou

Budapest

2016

Supervisor : Dr. Müller Linda
University of Veterinary Medicine
Department and Clinic of Reproduction

Table of contents

Introduction

The oestrous cycle of Canis lupus familiaris differs considerably from other species. The domestic bitch is a monoestrous, non-seasonal, spontaneous ovulator (Concannon, 2011). Which means that the oestrous cycle of the dog is characterized by a follicular phase with spontaneous ovulation, followed by a luteal phase of about seventy five days and later a non-seasonal anoestrus of 2–10 months (Concannon, 2011).

Pseudocyesis (PSC), pseudopregnancy or false pregnancy is a physiological syndrome that expresses symptoms similar to the post-partum signs (Gobello et. al., 2001b). The intensity of these symptoms is extremely variable among the individuals. Pseudopregnancy actually defines the species as the non-pregnant dog has a CL lifespan that often exceeds that in pregnant animals, (Gobello et. al., 2001b). The syndrome has a natural explanation as part of parental care. In group-living mammal species the term alloparental care exists which means care can be provided by adults other than the parents. Most canids that live-in packs demonstrate cooperative breeding, where submissive provide care to the offspring of the dominants, without reproducing themselves (Paul et al., 2014). Although there are no reports of cooperative breeding in groups as free-ranging dogs are known to live in stable social groups in which all adults have mating opportunities (Cafazzo et al., 2014), still they are descents of the grey wolf from which they evolved thousands of years ago (Gobello et. al., 2001b)

In conclusion being an atavism, PSC could have had some functional importance during evolution when non-bred female wolves had to nurse other females' litters (Jöchle, 1997). PSC is now a frequent phenomenon in domestic dogs and although its exact frequency is not known, it is estimated to be around 50±75% (Razzaque et al., 2008). Due to the variability among non-pregnant bitches, some dogs in mid and late metoestrus can express symptoms (overt PSC) whereas others may have no signs at all (covert PSC). This syndrome usually initiates with behavioral changes, such as restlessness, anorexia, decreased activity, aggression, licking of the abdomen, nesting, mothering inanimate objects, adopting other bitches' puppies. Next, follow the physical signs, such as weight gain, mammary enlargement, milk secretion, and sometimes abdominal contractions that mimic those of parturition (Gobello et. al., 2001b). Lactation does not start spontaneously, it is usually stimulated by self-nursing or by suckling of unrelated neonates (Razzaque et al., 2008). The syndrome expresses others symptoms as well as vomiting,

anorexia, diarrhea, polyuria and polydipsia, although these tend to be less common (Razzaque et al., 2008). When the condition is aggravated enough for these signs to become a clinical problem treatment is advised. Fortunately, complications of clinical PSC for example mastitis and mammary dermatitis are not regular and signs of PSC normally cease after 2±4 weeks (Gobello et. al., 2001b). Unfortunately though when a dog is susceptible the recurrence rate in successive oestrous cycles is quite high (Razzaque et al., 2008).

Diagnosis of the syndrome is done based on the observation of the clinical symptoms described above. As both late pregnancy and PSC present maternal behavior, pregnancy should always be considered as a possibility because owners may be unaware of the occurance of a mating. In case of doubt, ultrasound or radiography are methods that can be used to deliver a definitive diagnosis. Pyometra, which is another example of diseases of the luteal phase which can present anorexia, vomiting and depression should be ruled out by abdominal ultrasonography or radiography, a complete blood cell count and additional ancillary tests. What makes diagnosis of PSC challenging is the fact that it may coexist alongside other genital or extra-genital clinical problems (Gobello et. al., 2001b).

It was initially hypothesized that PSP was caused either by an overproduction of P4 or abnormal persistence of corpora lutea (Gobello et. al., 2001b). Later, it was suggested that the condition was related to increased concentrations of circulating prolactin (PRL) caused by an abrupt decline of P4 levels in the late luteal phase and the consequential loss of P4 negative feedback on PRL secretion (Allen, 1986). Overt PSP has also been observed after ovariectomy during luteal phase which is not surprising as this phenomenon is analogous to the fall in P4 levels subsequent to luteolysis before parturition. There are also experimental data supporting a causal role for P4 deprivation in the onset of canine PSP. Still the exact aetiophysiology is not completely understood so in this thesis the goal is to summarize the data published up to today.

The oestrous cycle of the bitch

The bitch has some features in relevance to reproduction that are unique for its kind. The female dog is monoestrus and the feature of seasonality appears to be in most breeds almost nonexistent (Gobello et. al.,, 2001b). The canine cycle is divided into four phases: a period of 5–20 day proestrus, a period of 5–15 day estrus, next a period of 50-80 day and last a period of anestrus lasting 80–240 days. Each of these phases contain a different leading hormone in dominant role altering the course of the follicle. So in proestrus the rise in estrogen ensures the follicular phase, then in the initial luteal phase there is a rise in progesterone and decline in estrogen, then the rest of the luteal phase, and last the breach between the seizing of luteal function and onset the of next cycle (Concannon, 2011). The main event of the cycle appears when the surge of the pre-ovulatory luteinizing hormone (LH) takes place (Concannon , 1977). Forty-eight hours after the LH surge, ovulation of primary oocytes occurs from incomplete luteinized follicle. In the following two or three days the oocytes in the oviduct go through the maturation process. Next after oestrus is the luteal phase (LP) called metoestrus. The central event here the progesterone (P4) secretion and function of the corpus luteum (CL). After day 30, P4 secretion is dependent on pituitary secretion of PRL and LH (Gobello et. al., 2001b). Administration of dopaminergic agonists, which lower the circulating PRL concentrations (Beijerink, ,2004) , has a luteolytic effect during the second half of the LP (Gobello et. al., 2001b). The P4 levels in unmated bitches are at a maximum 2 or 3 weeks after ovulation and then decline slowly to basal levels till the end of metoestrus. The long life of the CL in unmated dogs is due to the absence of release of any luteolys in from the uterus (Okkens et al., 1985). In the non-pregnant cycle there usually is sufficient stimulation of mammary tissue by P4 to make the metoestrus a physiologically distinct period. The end of metoestrus is defined by the drop of P4 concentration to basal levels (< 3 nmol/l). Evident mammary development may persist 1 or 2 months after P4 reaches this basal level (Gobello et. al., 2001b). Comparing the endocrine profiles between pregnant and non-pregnant bitches, there is an elevation of relaxin from day 25, and in PRL concentrations from day 30±35 that is found only in pregnant state (Gobello et. al., 2001b).

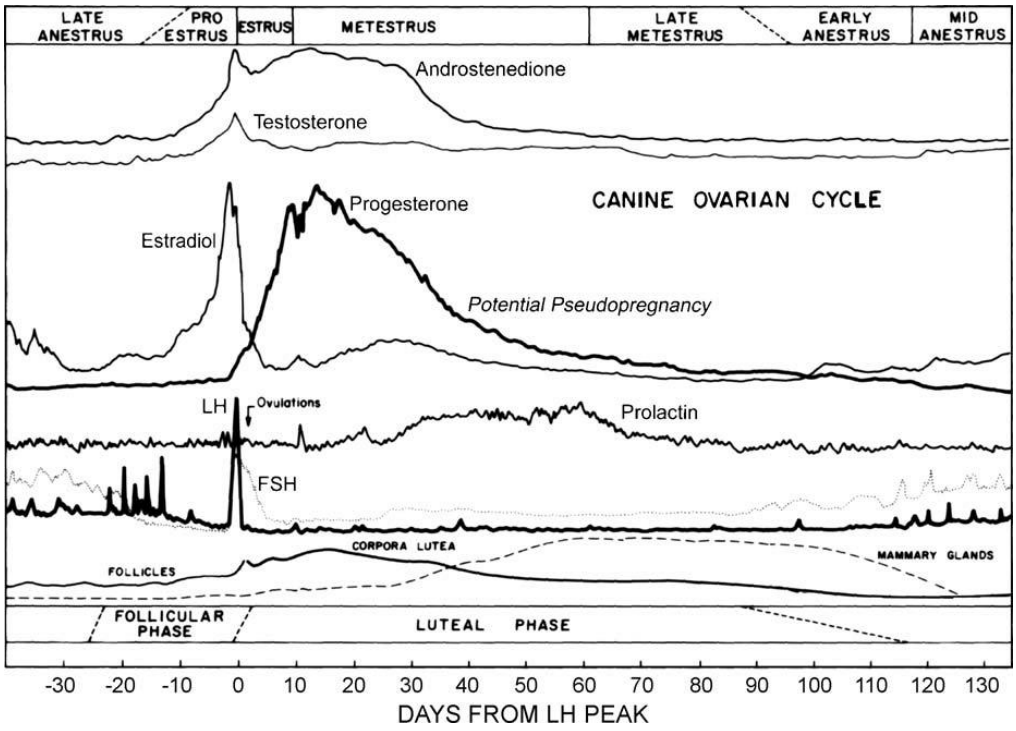

Figure 1. Schematic of regular changes in concentrations of reproductive hormones during the estrus cycle of the bitch. Most important features include a late proestrus peak in estradiol 1–2 days before the preovulatory LH peak; an increase in progesterone representing the preovulatory luteinization; the estrus phase that's characterized by falling estradiol concentrations and increasing progesterone concentrations and finally reaching peak values in early metoestrus around day 20–30; a decline in progesterone over a 4–8 week period after peak values showing slow luteal regression and lack of a uterine luteolytic mechanism; and, a small palpable increase in mammary size lasting for 3–4 months in every cycle. The transition from the metoestrus into anestrus is not exactly defined e.g. progesterone below 1 or 2 ng/ml, i.e., ~3–6 nmol/l, uterine histological repair. Also, its imprecise among dogs due to the different lasting luteal phases. Proestrus onset is also unclear, due to the different criteria and parameter used (i.e., vulval bleeding, estradiol increase, male interest) and among bitches with varying serosanguinous discharge and different rates of follicle development. Ovulation of immature oocytes occurs at 48–60 h after the LH surge has taken place (Concannon, 2011).

Endocrine profile of pseudopregnancy

Many studies have been made where researchers investigated the reproductive hormone concentrations comparing pregnant and/or pseudopregnant female dogs (Concannon, 1977). Collecting samples randomly during pregnancy and metoestrus the amount of unconjugated total estrogens and progestogens have been reflecting the expected physiological values (Chakraborty , 1987). On the contrary in the sampling done on pseudopregnant animals, the total unconjugated estrogen concentration was found to be mostly elevated. Other scientists like Smith and Mcdonald (1974) found that the concentration of LH in gestating bitches was like that of dogs presenting overt pseudopregnancy. Also, the peak progesterone concentration at 20 to 25 days after the LH peak was noticeably higher in pregnant animals compared with that in pseudopregnant bitches.

In another experiment done by Chakaborty (1987) ten labrador bitches were artificially inseminated with semen on the first day of their estrus, once every second day, three times in total. Next, six more dogs were inseminated in the same way with just pure saline. Seven out of ten animals that were inseminated with the semen conceived following with a physiological gestation. From the remaining three animals that failed to conceive one expressed signs of pseudopregnancy. As for the animals that were inseminated with saline five of them turned to be pseudopregnant. As for the cycles, the duration of proestrus was similar for animals that conceived and for animals subsequently determined to be pseudopregnant and ranged from 6 to 10 days for both groups. The mean duration of estrus for the pregnant animals was, however, significantly longer than that of the pseudopregnant animals. All animals were in estrus between Day -1 and Day 2 with respect to the day of peak preovulatory LH (Day 0). The mean time of initiation of behavioral estrus was 12 ± 2.4 hours following detection of the LH peak. There was no difference in this regard between the pregnant animals and those that were not inseminated with semen or failed to conceive (Chakraborty, 1987).

Analyzing the hormonal values Chokraborty and his colleagues (1987) found that LH had a much greater value one week after whelping for the pseudopregnant animals (5.21 ± 1.4 ng/ml) and not for the pregnant (1.44 ± 0.4 ng/ml), even though on the actual day of the whelping mean LH concentration for both was the same. Mean serum estrone value in the pregnant animals climbed to 68 ± 13 pg/ml on Day -1 from a concentration of 18 ± 3 pg/ml on Day -5. As for the

pseudopregnant animals, estrone concentration increased from 21 ± 4 pg/ml on Day -6 to 85 ± 16 pg/ml on Day -1. The mean peak serum of the estrone concentrations between the two groups was the same. Throughout the entire length of the pregnancy the mean estrone levels for the pseudopregnant bitches remained the same and appeared somewhat stable between Week 2 and whelping. On contrary, mean serum estrone for the pregnant animals raised again from the nadir on Day 4 and remained significantly above that of the pseudopregnant group throughout gestation except for the concentrations during Weeks 6 and 8 (Figure 2). The peak of the mean estrone concentration for the pregnant bitches (52.7 ± 13.2 pg/ml) was reached during midgestation so week 5. Serum estrone concentration for the pregnant animals declined from 42 ± 10 pg/ml on the day before whelping to 19.2 ± 3 pg/ml on Day 7 post whelping (Figure 2). No decline in serum estrone was observed in pseudopregnant animals during the corresponding period. By 1 week postwhelping, mean estrone concentrations were similar for both the pregnant and pseudopregnant groups of bitches. Analyzing the mean serum estradiol concentrations during proestrus showed a continuous increase analogous to that marked for estrone. Parallel mean peak concentrations of estradiol were observed on Day -1 for the gestating animals (63.7 ± 16.2 pg, Figures 2 and 3). Dogs undergoing pseudocyesis expressed mean serum estradiol concentrations drastically higher than those of gestating animals through Week 3 and lowest values were not reached until Week 5. After that serum estradiol levels of both groups remained alike. Checking the progesterone levels, it was observed that they stayed at basal levels (<l ng/ml) for all dogs until after Day 0. Mean progesterone level for the gestating dogs went from 0.5 ± 0.1 ng/ml on Day -1 to 2.0 ± 0.2 ng/ml on Day 1 and rose to zenith with a concentration of 10.5 on Day 6 (Figure 2). Mean progesterone concentrations of pseudopregnant bitches on Days -1 was 0.9 ± 0.3 ng/ml, on Day1, 2.0 ± 0.3 ng/ml, and Day 6, 20.0 ± 1.5 ng/ml. Both groups of dogs kept an inconsistent but high serum progesterone concentration for the next 9 week, levels for the pseudopregnant animals were extensively higher in comparison to those of the pregnant bitches during Weeks 1 to 6 (Figure 3) (Chakraborty,1987).

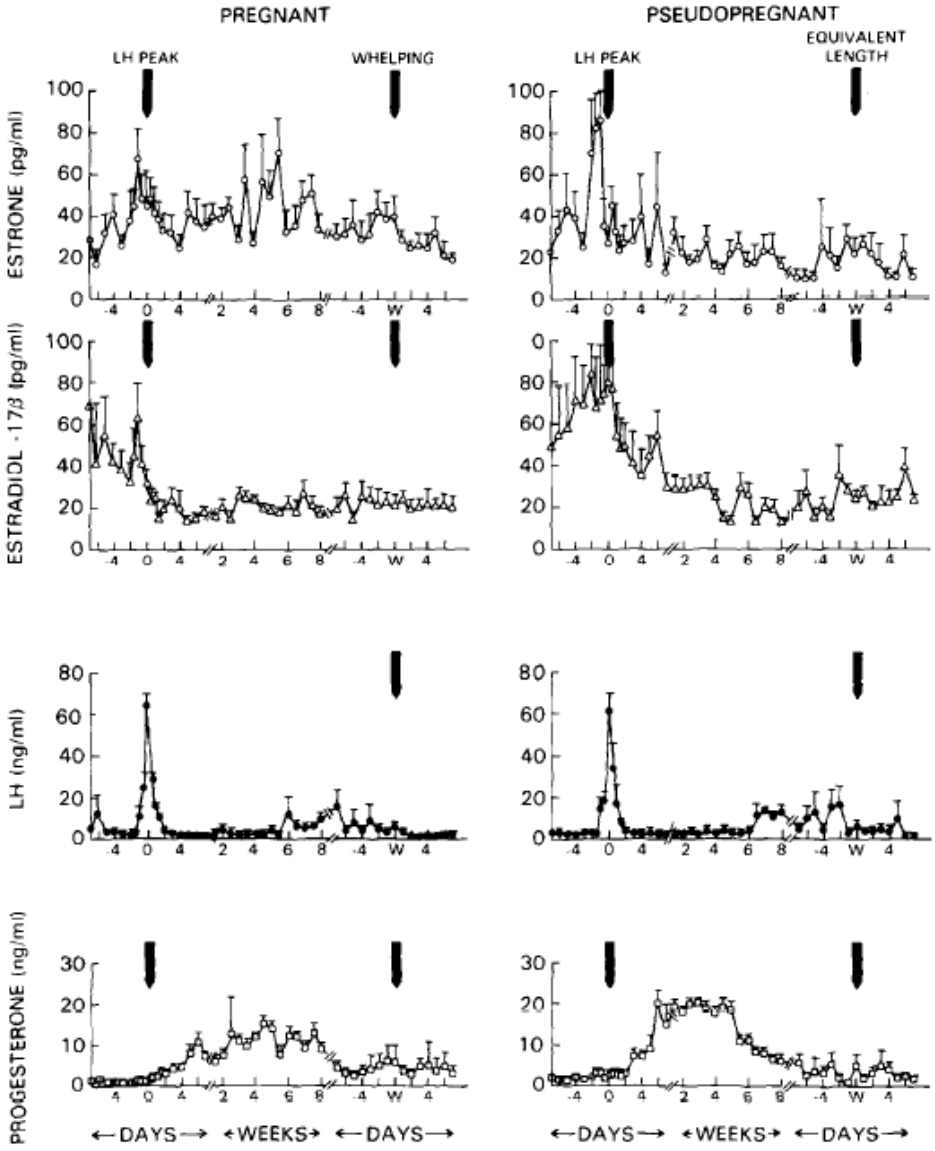

Figure 2. Demonstration of hormonal values during pregnancy and pseudopregnancy in the Labrador bitch. Estrone, estradiol, LH and progesterone during estrus. Day 0 represents the day of preovulatory LH surge and W the day of and the day of whelping (Chakraborty ,1987)

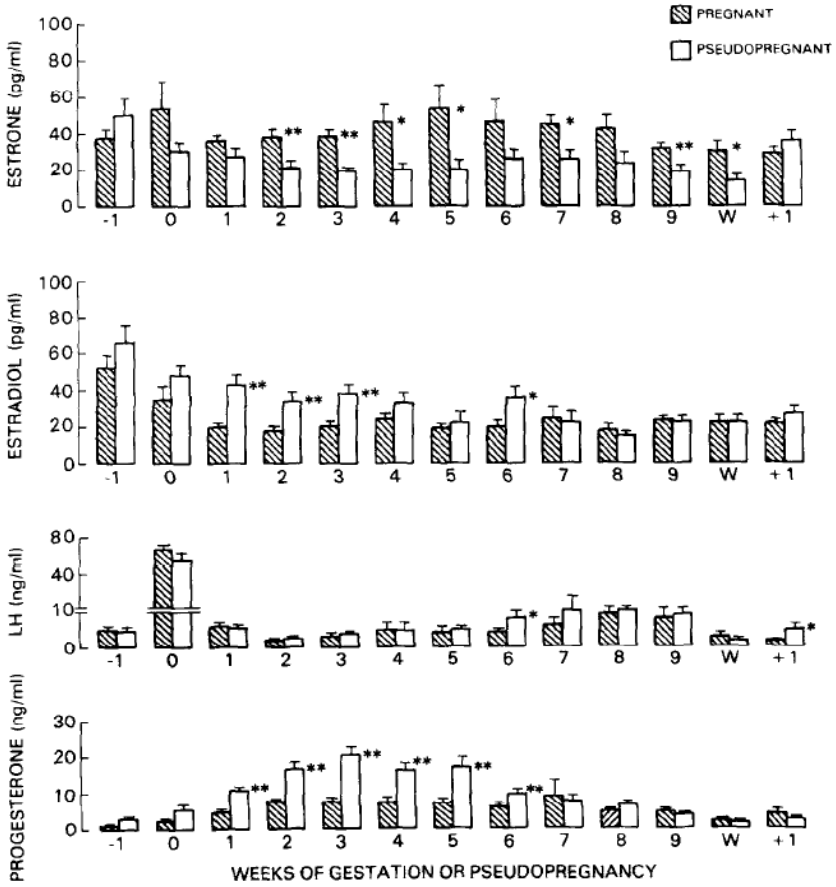

Figure 3. Comparison of mean serum concentrations of estrone, estradiol, LH and progesterone during estrus, gestation and pseudopregnancy (Chakraborty,1987).

The hormone prolactin and its secretion

Prolactin (PRL) is a peptide hormone produced by the lactotropic cells of the anterior pituitary. In addition, several extra-pituitary tissues produce PRL in a cell-specific manner and exert a local autocrine/paracrine response. The extra-pituitary sites include the breast, decidua, prostate, brainskin, fat and immune cells. In mammals, PRL is essentially involved in the control of reproduction (Ignacak et al., 2012). Particularly, in the domestic dog this hormone regulates gonadal function, mammary development and reproductive behavior (Jöchle, 1997). A great function of the hypothalamic nuclei is that it controls the level of several circulating pituitary hormones by releasing or inhibiting them (Egli et al., 2010). In succession, higher in hierarchy brain sites govern the hypothalamic nuclei, which have a centripetal role. The afferent stimuli to these centripetal sites can have neural or hormonal nature (Egli et al., 2010). Prolactin secretion is regulated by a continuous inhibitory tone of hypothalamic origin; whose main mediator is dopamine. Dopamine acts on D2 type dopamine receptors on the lactotropic cells (Egli et al., 2010). Prolactin secretion is further regulated by other numerous neurotransmitters and peptide factors. They may have either an inhibitory or stimulatory effect. The latter are histamine, vasopressin, oxytocin, thyrotropin releasing hormone (TRH), estrogens, GnRH and opiods (Ignacak et al., 2012). On the other hand, the mechanism of PRL secretion in extra-pituitary sites is not fully understood but seems to be cell type specific and is not necessarily dependent on dopaminergic system (Ben-Jonathan et al., 2008). The natural light from the surroundings causes PRL surges to the particular time (Bethea and Neill, 1980) of day possibly through actions of the suprachiasmatic nucleus (SCN) which is considered as the biological clock of all mammalians (Reppert and Weaver, 2002).

In female rat's scientists, have determined three different patterns of PRL secretion (Egli et al., 2010) (Fig. 4). Through research it's been proven that, the concentration of circulating PRL during the estrous cycle is small in normal cycling rats. Only one major PRL surge takes place at proestrus, which is parallel with LH during the cycle that has a time frame of four to five days (Egli et al., 2010) (Fig. 4A). Another category of PRL secretory cascade is initiated by a neuroendocrine reflex which is stimulated when the pups suckle on their mother (Fig. 4B). The moment pups start to suckle, PRL secretion escalades and, the level of its concentration escalades per the number of pups that are suckling. The third and last pattern answers to the

mating stimulus and lasts for ten days during pregnancy and twelve days during pseudopregnancy (Egli et al., 2010) (Fig. 4C). The pattern creates two PRL surges, one peaks the PRL concentration in the of the morning (0100–0300 h, nocturnal surge) and one in the evening (1400–1800 h, diurnal surge) (Freeman and Neill, 1972).

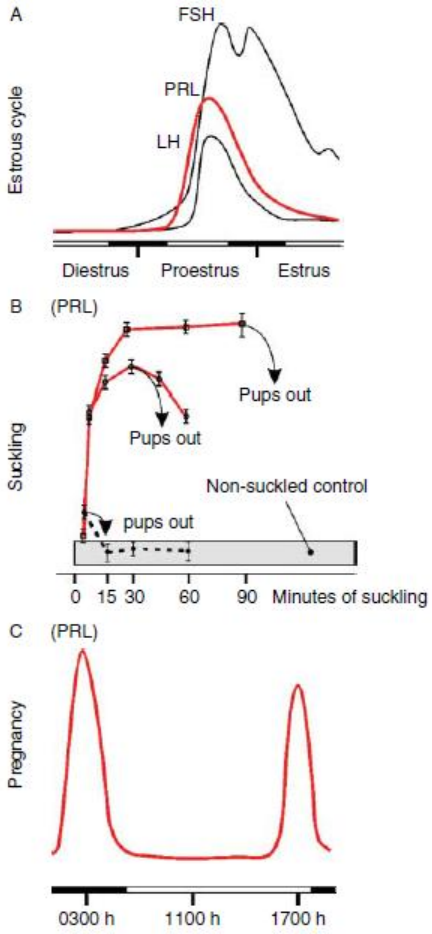

Figure 4. PRL secretory patterns of female rats. (A) Schematic illustration of the LH, FSH, and PRL levels during the estrous cycle in normal animals (B) Schematic representation of the PRL response of a nursing rat to the suckling stimulus of one, four, and six pups respectively. The PRL magnitude correlates with the number of suckling pups and the duration of suckling. Comparison of plasma profiles of oxytocin and prolactin following suckling in the rat. (C) Illustration of the PRL secretion pattern induced by mating (Egli,2004)

Dopamine and its crucial influence on prolactin

One of the neurotransmitter of the brain, Dopamine (DA), is the most important factor in PRL secretion as it has an inhibiting role (Ben-Jonathan and Hnasko, 2001). It is released by three neural groups of the hypothalamus: periventricular dopaminergic neurons, tuberohypophyseal neurons, and tuberoinfundibular neurons that belong to the arcuate nucleus (Egli et al., 2010). PRL also affects the level of DA as it stimulates DA neurons causing DA production creating this way its actual own negative feedback (Egli et al., 2010). In conclusion, PRL causes activation of DA neurons, which inhibits their own PRL secretion (Bertram, 2005). Given the above, scientists have speculated that the mechanism responsible for the PRL fluctuation in early pregnant or pseudopregnant rats could also depend on the previously mentioned feedback loop between hypothalamic DA neurons and pituitary lactotrophs (Bertram, 2005). Figure 5A, C, and E show the simulation of the PRL rhythm of early pregnant or pseudopregnant rats estimated by the mathematical model that combines three equations, for further details on the model, see Egli et al. (2004). Another factor influencing PRL is oxytocin, proven by the fact that ovariectomized rats, in which the DA receptor is blocked, an oxytocin antagonist could block the PRL secretion (Egli et al., 2010). This leads as at the assumption that oxytocin compiles PRL-releasing properties. Oxytocin in turn is triggered by a mating stimulus (Egli et al., 2005). The actual concentrations of oxytocin, PRL and DA calculated in the animals are demonstrated in Fig. 5B, D, and F.

As previously mentioned earlier the suprachiasmatic nucleous is the mammalian master clock as it controls all the activities related to the circadian rhythm (Reppert and Weaver, 2002). Thus to that its neurons also influence DA and oxytocin neurons. (Egli et al., 2010). Vasoactive intestinal peptide (VIP) fibers which have the SCN as a starting point innervate the arcuate nucleus and the periventricular nucleus exciting the DA and the oxytocin neurons respectively (Egli et al., 2010).

Analyzing the pattern, the first PRL surge stimulates the hypothalamopituitary PRL–DA system and activates the PRL secretion as stated earlier (Fig. 5A). Further the VIP in the morning creates an inhibitory effect on DA activity. Therefore, the elevated VIP value produced by the suprachiasmatic nucleus of the hypothalus (Fig. 5C, red) decreases DA activity (Fig. 5E). The drop of DA decreases the inhibitory effect to the lactotrophs, fascilitating the occurrence of an early morning PRL surge (Fig. 5A). Thus, VIP promotes the event of the nocturnal PRL surge,

and simultaneously connects the PRL rhythm to a 24h period. Lacking the periodic VIP secretion, the morning PRL surge would stray due to the free-running DA–PRL rhythm. Additionally, the VIP creates PRL surges of different intensity (Fig. 5A). PRL surges of different magnitude are atypical finding in early pregnancy/pseudopregnancy in rats (Fig. 5B; Smith et al., 1975). The diurnal PRL surge which results in a peak early evening in this mathematical model is exclusively induced by oxytocin (Fig. 5C and D). Despite the fact the mathematical model used combines just three equations, it is still proficient in mimic the core aspects of the PRL secretory rhythm of early pregnant/pseudopregnant rats (Fig. 5B, D and F). Moreover, it proposes potential chronological releasing patterns for the PRL realizing and decreasing factors oxytocin and DA, in addition to the timing signal VIP, which are essential for developing the PRL rhythms (Fig. 5A) (Egli et al., 2010).

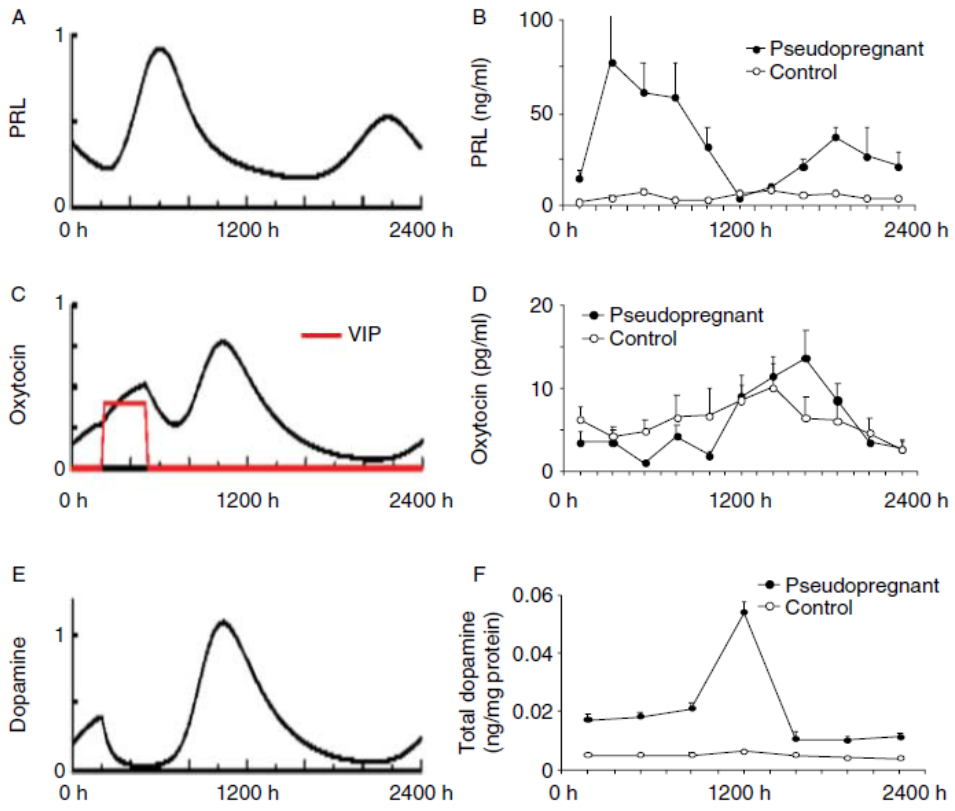

Figure 5. Comparison between the simulation and the experimental data collected from rats. The simulation of PRL secretion (A) is analogous to the measured fluctuation in pseudopregnant animals after cervical stimulation (B, filled circles). On the contrary, PRL concentrations of not stimulated animals lack fluctuation (B, open circles). Likewise, the calculated (C) and the driven oxytocin release patterns (D) are similar. However, the calculated oxytocin surge emerges just before the values peak in rats. The VIP timing signal is also represented by C (red). Calculated dopamine values (E) are in line with the actual fluctuation measured in pseudopregnant rats (F) (Egli et al., 2010).

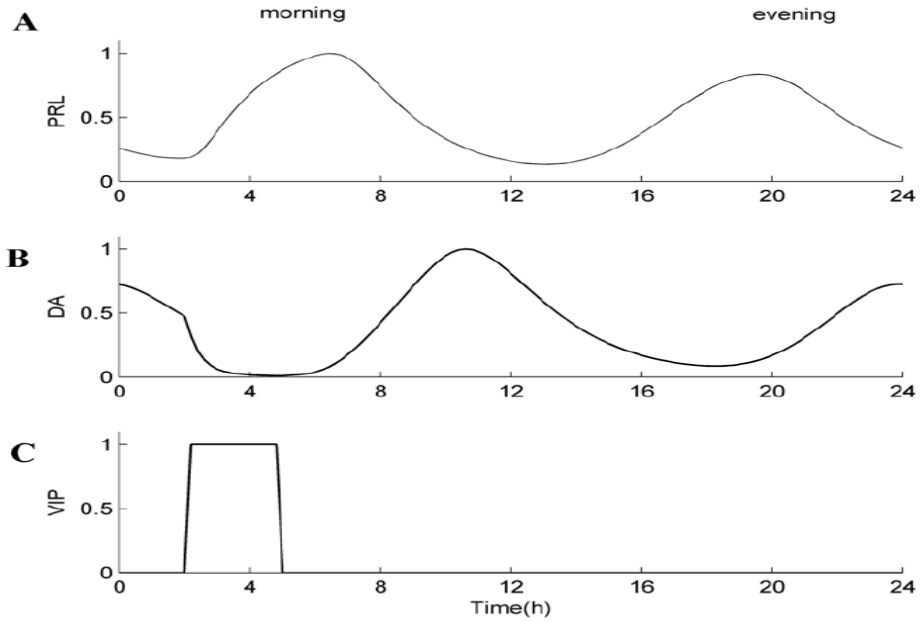

Figure 6. VIP entrains PRL rhythm and increases morning surge. (A) VIP entrains the PRL rhythm so that first surge occurs at the same time each day. VIP also causes the magnitude of the morning PRL surge to be enhanced relative to the afternoon surge. (B) VIP inhibits DA in the morning, advancing the time of occurrence of the first PRL surge. (C) VIP is treated as step function that is elevated for 3 h early each morning. The pulse of VIP (Fig 6C) from 2 until 5 a.m. reduces the DA activity (Fig 6B). This decline in DA accelerates the PRL release. The nocturnal surge of PRL appears earlier in the morning. Thus, the VIP sets the phase of the morning surge to the same time every day, making the PRL rhythm period exactly 24 hours. Another result of VIP addition is that the morning surge now is larger than the afternoon one. Therefore, the VIP provides the time of day signal as well as the unique asymmetric shape for the PRL rhythm (Toporikova, 2007).

Prolactin secretion pattern during the luteal phase

Even though in rodents PRL is a luteotrophic hormone, its role in the regulation of the corpus luteum differs greatly in mammalian species (Niswender et al., 2000). There are reports regarding patterns of plasma prolactin levels in the female dog during gestation, and lactation and during the different phases of the oestrous cycle and anoestrus. Kooistra and Okkens (2001) ,in his investigations observed an raise in plasma prolactin level in pregnant bitches specifically in the last week of pregnancy, while Onclin and Verstegen (1997) ,according to Kooistra and Okkens(2001) ,noticed an obvious increase from the middle of gestation till the whelping (Kooistra and Okkens,2001). Onclin and Verstegen specifically recorded a raise in plasma prolactin levels, by measuring daily ten pregnant bitches, and found that it was altered from those in 10 non-pregnant bitches around day 35 following the pre-ovulatoy LH peak (Kooistra and Okkens,2001). In their investigation mean plasma prolactin values peaked at 42 ng/ml on the day of parturition, dropped to 16 ng/ml for the next 24±48 h and then suddenly increased again to peak at around 40 ng/ml. In the time of lactation, high plasma prolactin level was measured, but with big discrepancies. Post weaning, approximately 107 days after the LH peak, prolactin levels subsided and assimilated to that of the non- gestating animals by day 120.

Plasma prolactin levels in non-pregnant bitches appear to be lower than in pregnant animals, at least in the last part of the luteal phase (Kooistra and Okkens, 2001), but data from various investigations concerning the luteal phase differ. In the experimental research of Onclin and Verstegen (1997) the plasma prolactin values in non-gestating beagle bitches (mean concentration 5 ng/ml) present no serious variation during the observation period from day 15 before the pre-ovulatory LH peak up to day 135 after(Kooistra and Okkens, 2001). A small and trivial increase was measured at about day 70. In Kooistra's research (2000) the prolactin plasma concentrations were measured in a time frame of 12 hours at 10 minutes intervals with a mean of 19 for luteal phase 1, 38 for luteal phase 2, 57 for luteal phase 3, 78 for luteal phase 4 and 142 during mid-anoestrus days after the predicted day of ovulation. Throughout all luteal phase and during mid-anoestrus, the nature of prolactin secretion was done in an a irregular manner with sporadic distinct boosts, suggesting a pulsatile release of prolactin. Comparing the mean basal plasma prolactin values during the different luteal phases it was observed that in luteal phase 4 (5.1 ng/ml) concentration was considerably higher than those during luteal phase 1 (2.5 ng/ml), luteal phase 2 (3.0 ng/ml) and mid-anoestrus (2.0 ng/ml). The mean basal plasma prolactin level

at luteal phase 3 (4.1 ng/ml) was notably elevated compared to that during mid-anoestrus. Correspondingly, the mean area under the curve (AUC) for prolactin above zero was radically advanced during luteal phase 4 in comparison to luteal phase 1, luteal phase 2 and mid-anoestrus. Also the mean AUC for prolactin was much bigger in luteal phase 3 compared to mid-anoestrus. In conclusion development of the luteal phase was proven to be correlated with an enhancement in basal plasma prolactin levels and AUCs for prolactin above the zero-level (Kooistra et al., 2000).

Physiological effects of prolactin

Apart from the discussion on the importance of increased prolactin secretion during succession of the luteal phase also the role of prolactin in mammogenesis and the luteotrophic properties of prolactin are worth mentioning. Research conducted using prolactin receptor-knockout mice have exposed that lobuloalveolar development in the mammary gland is driven almost solely by prolactin (Brisken et al., 1999). As growth and development of the alveoli mostly take place during the more advanced phases of development of the mammary gland, so during the second part of the luteal phase in the bitch, lobuloalveolar growth coexists with increased prolactin release in this species (Kooistra and Okkens, 2001). Experiments run on hypophysectomized dogs proved that pituitary luteotrophic support is crucial for the second stage of the luteal phase in the bitch (Okkens et al., 1986).The corpora lutea is prolactin-dependent exactly the time of the elevated prolactin secretion. Another factor influencing PRL and its secretion is LH which in addition to its direct effect on lutein cells, possibly mediates in the PRL-dependent P4 production (Kowalewski, 2014). Then again, PRL did not cause the generation of LH and the inhibition of PRL release did not alter LH levels (Kowalewski, 2014). Also PRL was not found to immediately trigger plasma P4 (Kowalewski,2014). As a result, despite the fact that PRL seems to be the necessary luteotrophic factor from the mid-luteal phaseand after , both during gestation and in the non-gestating canine cycle, its role is mostly supporting CL function and/or postponing luteal regression, as opposed to actively stimulating P4 creation (Kowalewski ,2014).

Prolactin receptor isoforms

The canine PRL receptor (PRLR) cDNA responsible for the extra- and trans-membrane domains of the different PRLR isoforms of other species was recently sequenced and cloned (Kowalewski et al., 2011). These different PRLR isoforms have in common an extracellular and a transmembrane domain, but are different in structure and length of their cytoplasmic domain, and hence are categorized into the long form (PRL-RL) and the short form (PRL-RS) (Kowalewski et al., 2011).

The PRLR belongs to the class 1 cytokine receptor superfamily in which the intrinsic tyrosine kinase activity is absent (Saangeta and Halperin, 2014). The expression of PRLR was investigated in canine CL from non-pregnant and pregnant animals demonstrating a time-dependent expression pattern. Also the protein values have been measured at the beginning of the CL phase and the considerably decreased concentation right before or during luteolysis (Kowalewski et al., 2011). This cycle phase-dependent expression of PRLR implies a functional interdependence with circulating P4 serum and proposes that PRLR may possibly be an upstream mediator in luteal steroid regulation in the dog (Kowalewski, 2014). In rodents, both PRL-RL and PRL-RS isoforms are co-expressed in luteal, granulosa, and interstitial, cell types throughout the estrus cycle, with PRL-RL being the most prominent one (Saangeta and Halperin ,2014).

PRLR expression levels differ along the estrus cycle as well as stages of pregnancy. Both of the isoforms, reached their zenith in mRNAs level at proestrus, displaced by a decrease during estrus, and then a shot back to maximal levels by end of diestrus and early stages of proestrus (Saangeta and Halperin ,2014). This decline in PRLR levels most likely plays a crucial part in debilitating PRL actions in many periovulatory events for different ovarian cell types (Saangeta and Halperin ,2014) .Furthermore, sustaining high PRLR levels in late diestrus is in relation with the need for PRL to keep progesterone production in order to prepare for pregnancy or pseudopregnancy. It is observed that in preovulatory granulosa cells there is a quick and sudden elevation in PRL-RL expression, in comparison to early follicles and that suggestes that PRL palys an important role in mature follicles (Saangeta and Halperin, 2014). Investigations in other species as well suggest that the need of progesterone production is in accordance with an increase in PRLR expression (Saangeta and Halperin, 2014).

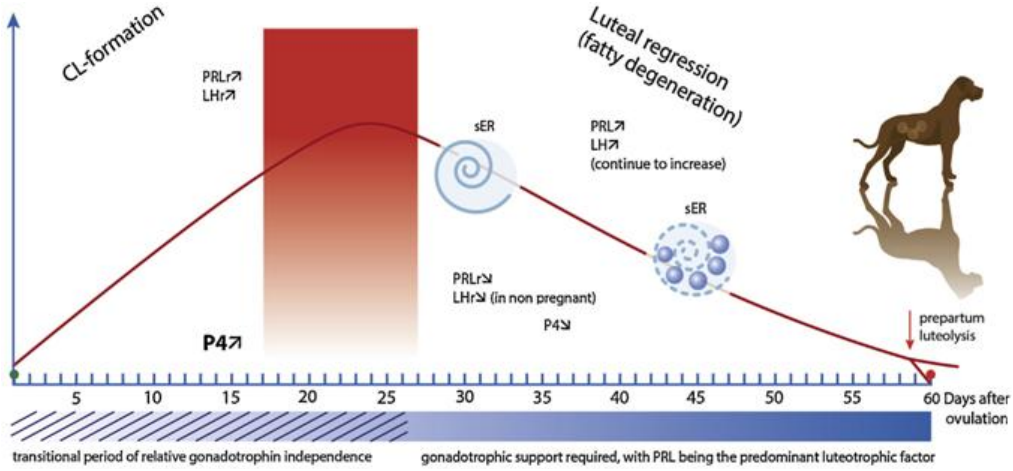

Figure 7. A schematic representation of suggested hormonal mechanisms governing canine corpus luteum (CL) function. PRL and LH are luteotrophic factors, with PRL being the predominant one (Kowalewski, 2014).

Prolactin and its role in pseudopregnancy

In overtly pseudopregnant bitches measuring plasma prolactin, high concentrations can be found. In six overtly pseudopregnant Afghan hounds the mean plasma concentration of prolactin was 35 ng/ml, which was significantly higher than that of the mean plasma prolactin concentration (6 ng/ml) in an earlier part of the luteal phase of three, non-pseudopregnant Afghan hounds (Gobello et. al., 2001b). Although the pathogenesis of an overt pseudopregnancy is still poorly understood, a rapid decline in the plasma concentration of progesterone may be a precipitating factor. The association of a strong increase of prolactin release and a decrease of plasma progesterone concentrations has been demonstrated in overtly pseudopregnant bitches (Gobello et. al., 2001b). Correspondingly, ovariectomy performed in the luteal phase often induces overt pseudopregnancy. Also, the fact that progestagen treatment can be effectively used to terminate overt pseudopregnancy in dogs, although it is not advised because of its side-effects, indicates that there is an inverse relationship between concentrations of progesterone and

prolactin. Moreover, administration of a progesterone-receptor antagonist to pseudopregnant and pregnant bitches causes plasma prolactin levels to rise sharply (Gobello et. al., 2001b).

The increase in PRL pregnancy is in correspondence to the placental release of relaxin which begins between days 25 and 30, and continues until parturition suggests a stimulatory role of PRL secretion and maybe justifying this way the higher P4 concentration observed during pregnancy (Kowalewski, 2014).

Other conditions connected to prolactin

Prolactin has also been linked to cancer. According to scientists in the 70s, hyperprolactinemia was proven to induce mammary carcinogenesis in mice and rats (Michel et al., 2012). As was previously noted in plethora of mammalian species, PRL is involved in proliferation and differentiation of normal mammary epithelium and in stimulating postpartum lactation. Mammary tumours represent the most common neoplastic disease of the intact female dog accounting for approximately 50% of all tumours in bitches (Sorenmo, 2003). Whereas the influence of sexual steroids on breast cancer (BC) development in dogs has been studied, very little is known about the influence of PRL (Michel et al., 2012). Now latest research demosntrates the importance of PRL in human BC development and progression. Its been proven that PRL drives the cell growth of both benign and malignant neoplasties in BC *in vitro* as well as *in vivo*. (Michel et al., 2012). The signaling cascade triggered by PRL is additionally derailed by impaired PRLr turnover. For example in healthy mammary epithelium PRL promotes the downregulation of its own receptor, but PRLr in BC cells is degradation- resistant, thus amplifying PRL signaling (Michel et al., 2012). BC cells expressing PRLr are characterized by higher proliferative and invasive properties, whereas suppressed PRLr expression radically declines carcinogenic properties. Sporadic publications proposed a tumour promotor role also in the dog. Some investigations show a decrease in PRLr expression in canine mammary tumor compared to healthy mammary tissue (Michel et al., 2012). So because of the unjustified mammary tissue proliferation in both carcinogenesis and pseudopregnancy studies have been made aiming to investigate the possible correlation between the two. Veronesi (2003) demonstrated that pseudopregnancy has no influence on the onset of mammary tumours in the bitch. There are contradicting views on this topic supports the hypothesis of an increased

predisposition to onset of mammary tumours in bitches with pseudopregnancy, with a much elevated risk as recurrence of pseudopregnancy and age of the bitch increased. In conclusion, the results of this study show that there is no relationship between tumoural class and pseudopregnancy in bitches with mammary tumours (Veronesi et al., 2003).

Scientific investigation on the significance of prolactin in pseudopregrancy

Although the importance of PRL in the appearance of pseudopregnancy is widely accepted, many different scenarios about the aetiology were suspsected. Initially it was believed that pseudopregnancy was a result of either by an elevation of P_4 or longer lifespan of corpora lutea (Allen, 1986). Later in time clinical signs of pseudopregnancy were believed to be connected to an elevation of circulating PRL caused by a sudden drop of P_4 levels near the end of luteal phase (LP) and the resulting loss of P_4 negative feedback on PRL secretion (Allen, 1986; Smith and Mc Donald, 1974).

Overt PSP has also been observed after ovariectomy (OVX) during LP (Gobello et. al., 2001c). Although its incidence has not been documented, it is generally accepted to be fairly low. Actually, the appearance of PSP after OVX is not a surprise (Gobello et. al., 2001c). This phenomenon is very much alike to the drop of P_4 levels after luteolysis before parturition. Also experimental data exist supporting a causal role for P_4 deprivation in the onset of canine PSP. Thus, in a study in which the antigestagen mifepristone was administered to 14 bitches at 3 different time points during the LP, all of the animals presented mammary hypertrophy (Gerres and Hoffmann, 1994). It has also been reported that hysterectomy, performed during the first half of the LP, caused a decrease in P_4 levels and the appearance of PSP in bitches (Gobello et. al., 2001c). Unfortunately, in 2 other studies with similar experimental design mammary status was not reported (Gobello et. al., 2001c). Despite the above information, recent reports challenge the theory that puts P_4 drop at the center of PSP. Thus, appearance of PSP was reported in bitches ovariectomised (OVX) 6 months after oestrus, a time at which P_4 levels are already basal (Gobello et. al., 2001c).In order to comprehend how do really PRL and P_4 play a role in the physiopathology of PSP, Gobello and her colleagues (2001c) decided to use 24 dioestrous OVX as a model to study the genesis of PSP in the bitch. This model allowed them to follow the dynamics of serum PRL and P_4 after OVX and correlate these hormone changes with the

appearance of PSP or lack of it, in pure- and cross-bred bitches. Eighteen dogs were devided into two groups. First group consisted of 11 animals OVX (OVXG) on day0 and the second of 7 intact animals used as a control group(CNTG). On day -1 of the experiment PRL and P_4 levels of the 18 female dogs showed no significant differences among groups. On day 7 post OVX, the dogs were checked for pseudopregnancy (PSP) signs. Out of the 11 OVX 4 presented overt pseudopregnancy, all of which had a history of the syndrome. On the same day the P4 level was measured and it was found that CNTG group had a higher level and a smaller percental change than OVXG. Measuring the P4 drop it was found to be quite similar between PSP group (PSPG) and nonPSPG. Next checking the PRL levels, it was found that they had increased for all PSPG between day -1 till day 7. Experiments proved that after OVX, PRL in pseudopregnant bitches reach levels which overlap with those of the non pseudopregnant, are in accordance with studies reporting lack of major differences in PRL levels between surgery-induced and non pseudopregnant animals (Hoffmann et al., 1992) and an overlap of PRL serum levels between pseudopregnant and non pseudopregnant bitches post spaying (Gobello et. al., 2001c). Elevated PRL levels do not appear to be required for PSP presence as recommended by a report of low PRL levels in 2 spayed pseudopregnant bitches (Harvey et al., 1997). A rather interesting finding is that only those bitches that underwent a rise in serum PRL after OVX developed PSP and that this syndrome only appeared in predisposed animals. For that reason, it is concluded that only in predisposed dogs would a sudden in P_4 be able to stimulate a considerable increase in PRL concentrations, which in time would trigger the typical signs of PSP. So, a fall in P_4 does not appear to be, sufficient to induce PSP in all female dogs. From the present results the investigators concluded that in the bitch, the key factor in the genesis of PSP appears to be the marked rise in PRL levels that occurs in PSP-prone animals after a abrupt fall in P_4 as a sudden decline of P_4 levels did not lead systematically to PSP in all bitches (Gobello et. al., 2001c).

Another study was made by Gobello in order to clarify the role of PRL and P4 in canine PSP (Gobello et. al.,,2001d). The objectives were: to define the alteration of PRL and P4 serum values in overtly pseudopregnant (PSPT) dogs prior and throughout pharmacological blockade of PRL release with two dopaminergic agonists of diverse specificity and a placebo (PL); and to connect PRL with symptoms of PSP, previous to and during treatments and to evaluate the clinical performance and adverse effects of the treatments. A total of 30 overtly PSPT bitches, cross and pure-bred, were used in the conduction of this experiment. The bitches were randomly

put into three groups of 10 animals each: PL which was treated with a PL (with food), once daily for 7 days; BR, treated with 7.5 g/kg BW of bromocriptine, twice daily for 14 days; and CA, treated with 5g/kgBW of cabergoline, once a day for 7 days (Gobello et. al., 2001d).

Grade	Clinical signs
0	Normal mammary glands
I	Mammary enlargement + serous secretion
II	Mammary enlargement + milk secretion[a]

[a] Frequently associated with behavioral changes.

Table 1. Grades of intensity of clinical signs of PSP (Gobello et. al.,, 2001d)

The treatment started on day 0 and on days 1, 7 and 14 all the animals went through physical examination in order to be evaluated into grades (Table 1). Overt PSP was classified as grade II, complete remission as grade 0 and an in-between state was considered grade I. All dogs started in grade II in the beginning of the experiment (day 0). Mammary growth was determined as compared with the normal mammary size that exists during the anestrous period. Serum PRL and P4 concentrations (ng/ml) of the bitches on day 1 were 17.70±2.05 and 1.13±0.13, respectively. No real discrepancies among groups were noticed for PRL or for P4 values on the same day(Figs. 7 and 8, respectively). Serum PRL and P4 levels declined along the course of the experiment (day effect); Figs. 7A and 8A, respectively). Investigators observed that, serum PRL concentrations were higher in the PL group compared with treated group (BR and CA). (Fig. 7A,). A week later a significant difference in these percentages was found between DA treated and PL animals. In contrast, no important alterations were found in the percent variation in PRL among the BR and the CA animals (Fig. 7B) or in P4percentage variation among all groups for the same week (Fig. 8B). A fact worth mentioning is the connection between the same hormones that was suspected when days 7 and 14 were considered for the BR and CA animals together, that didn't agree with the PL group of animals. Whereas in the PL group, PRL values and grades of clinical signs were not linked on days 1, 7 and 14; in the BR and CA groups they were significantly interrelated on days 7 and 14. Concentration of P4 and the grade of clinical signs were also connected on the same days in DA-treated animals.

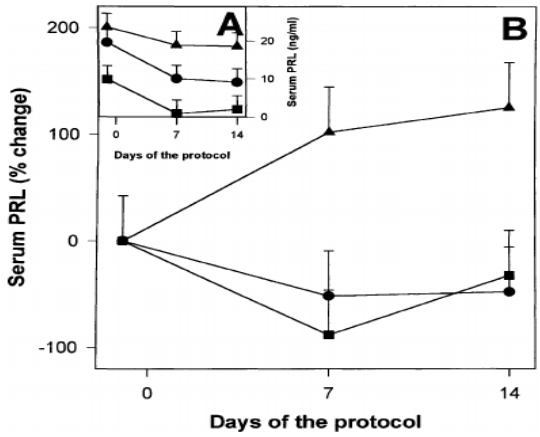

Figure 7. Serum PRL expressed as absolute values in ng/ml (a) or as % change for each of the 4 days of the protocol to day –1 (b). Groups PSPG (n=4), non PSPG (n=7) and CNTG (n=7) are 5 demonstrated with circle, square and triangle shape, respectively. The bars on the shapes symbolize 6 SEM. On day 7, the PSPG had bigger percent alteration of PRL concentration than nonPSPG (Gobello et. al.,,2001d).

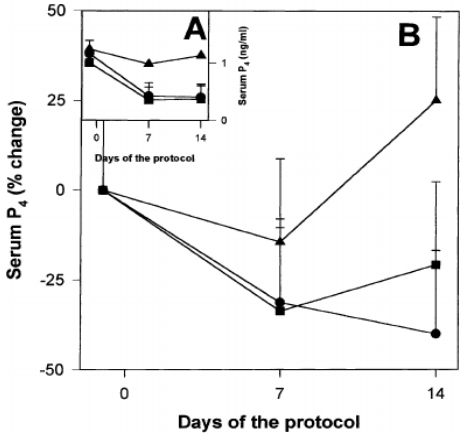

Figure 8. Serum P_4 expressed as absolute values in ng/ml (a) or as % change for each of the days of 13 the protocol to day –1 (b). Groups PSPG (n=4), non PSPG (n=7) and CNTG (n=7) are demonstrated with14 circle, square and triangle shapes, respectively. The bars on the symbolize SEM. Ovariectomised 15 dogs (PSPG+nonPSPG) had lower P_4 levels ($p<0.05$) and bigger percent alteration of P_4 (than 16 CNTG (Gobello et. al.,,2001d).

In conclusion the evidence of a positiveconnection among PRL concentrations and clinical symptoms that surfaced through the obligatory blockade of PRL secretion by DA demonstrates the key role of PRL in PSP etiopathology. However the lack of connection during spontaneous involution of PSP in the PL group shows that total PRL concentrations do not utterly explain the problem. In a earlier study no major variation were found in PRL values between PSPT and non-PSPT animals (Hofmann ,et al 1992). The existence of two spayed PSPT animals with small PRL concentrations and an overlap of PRL serum concentrations among PSPT and non-PSPT bitches post spaying (Gobello et. al.,,2001d) were stated in other investigations. These findings suggest that total PRL concentrations do not completely explain the etiology of PSP in the bitch. In our study abrupt changes in serum PRL, as produced by DA, seemed to be more important in cessation of pseudopregnancy signs than total PRL concentrations. The presence of elevated values of receptors in target organ or increased peripheral sensitivity to PRL as has been considered earlier could be another reason why the inconsistency among intensity of clinical symptoms and PRL concentrations exists. Lately ,the occurrence of PRL heterogeneity on molecular level was reported in diestrous bitches (Gobello et. al., 2001a). Variations in the immunoreactivity and bioactivity of canine PRL could explane , at least up to one point , the inconsistency among immunoassayable circulating concentrations of canine PRL and the presense of a biological response, specifically, pseudopregnancy. The small P4 serum values reported on day 1 verified that all the dogs were in brief period of approximately 30 days. This conformed to the late diestrous or early anestrus and most likely indicates the dissimilar lifespan of the LP in the animals investigated. This finding along with the lack of correlation between PRL and P4 suggests the absence of an inverse relationship in the secretion patterns of this two hormones and proves that P4 does not play any fundamental role in the maintenance of pseudopregnancy.

Discussion

The pseudopregnancy of the bitch is a syndrome commonly present in non-gestating dogs after 6-12 weeks of estrus and characterized by the signs of vulvas distension, lactation and mammary gland enlargement (Zubair**,** 2014**)**. Its a state obligatory for all non-gestating females in these species, which secures their ability of tending for and even nursing the young (Jöchle, 1997). Females that were pregnant or pseudopregnant are prepared to nurse and tend for whelps simultaneously. As for males the seasonally peaking PRL blood values seem to even over social tensions between them and ensure their essential participation in the care of the litter (Jöchle, 1997).

Treating with potent PRL inhibitors, mostly dopamine agonists like bromocriptine, metergoline and cabergoline, have proved that PRL is the luteotropic hormone from day 30 of pregnancy onward and that PRL **is** essential for the preparation of the mammary glands for lactation, and its maintenance. Also being essential luteotrophic factor makes it the hormone mandatory for maintaining progesterone secretion during the normal lifespan of the corpora lutea (Jöchle, 1997).

Research has been conducted on the expression of PRLR in CL of the dog from non-gestating and gestating animals proving that the expression has time-dependent manner (Kowalewski, 2014). This connection to the different cycle stage implies a possible functional interdependence with circulating P4 levels and suggests that PRLR could act as an upstream regulating factor in luteal steroid regulation in dog. So, these PRL-inhibitors are in use for induction of abortion aftermid-gestation, for the treatment of overt pseudopregnancies and to stop unwanted lactation (Kowalewski, 2014).

Earlier in time clinical signs of pseudopregnancy were believed to be connected to high concentrations of circulating PRL caused by a sudden decline of P_4 levels in the late luteal phase and the consequential loss of P_4 negative feedback on PRL secretion (Allen, 1986). In an experiment with six overtly pseudopregnant Afghan hounds the mean plasma concentration of prolactin was significantly higher than that of the mean plasma prolactin concentration in an earlier part of the luteal phase of three, non-pseudopregnant Afghan hounds (Gobello et. al., 2001b). The association of a strong increase of prolactin release and a decrease of plasma progesterone concentrations was demonstrated in overtly pseudopregnant bitches (Gobello et. al., 2001b).

Overt pseudopregnancy has also been observed after ovariectomy during luteal phase (Jöchle, 1999). Actually, the appearance of PSP after OVX is not a surprise. This phenomenon is very much alike to the drop of P_4 levels after luteolysis before parturition. So scientist conducting an investigation concluded that in the bitch, the key factor in the genesis of pseudopregancy appears to be the marked rise in PRL levels that occurs in pseudopregnancy-prone animals after a abrupt fall in P_4 as a sudden decline of P_4 levels did not lead systematically to pseudopregnancy in all bitches.

Another study was done by Gobello (2001d) to define the change of PRL and P4 serum level in overtly PSPT clinical cases prior to and during inhibition of PRL secretion with two dopamineric agonists of dissimilar specificity and a placebo. Results suggested that total PRL concentrations do not completely explain the etiology of PSP in the bitch. In the study sudden changes in serum PRL values, as produced by dopaminergic agonists, seemed to be more important in cessation of PSP signs than total PRL concentrations (Gobello et. al., 2001d).

Prolactin has also been linked to cancer. So because of the unjustified mammary tissue proliferation in both carcinogenesis and pseudopregnancy studies have been made aiming to investigate the possible correlation between the two. Researchers proved that there is no significant relationship between classes of lesions and presence of pseudopregnancy before tumoural onset (Veronesi, 2003).

In the dog, a strong inter- and intra-individual variability of PRL level was reported in 24-h serum measurments however, this was not attributed to a circadian rhythm. So dogs are very individually unique as to their prolactin profile. To summarize, till today only the increase of PRL in PSP-prone dogs has been linked to the syndrome but still the exact physiology behind it has not been completely unraveled.

Summary

Pseudocyesis or else pseudopregnancy is a physologicak syndrome in the dog which serves the survival of the canine pack .It conists to symproms similar to postpartum signs like mothering objects, mammary enlargement and even lactation. It was initially hypothesized that pseudopregnancy was caused by increased concentrations of circulating prolactin caused by an abrupt decline of progesterone levels in the late luteal phase and the consequential loss of progesterone negative feedback on prolactin secretion. Due to the prolactin leading role many scientists have condected investigations in order to understand this hormone in depth. Experiments on mice have demonstrated the prolactin secretion pattern during non pregnant healthy, pregnant and pseudopregnant animals. Next trying to comprehend the mechanism of the fluctuating concentration level in pseudopregnant bitches dopamine,a prolactin inhibiting factor was used in a mathematical model that proved PRL rhythm during pseudopregnancy. Apart from the investigation on the importance of increased prolactin secretion also the role in mammogenesis is worth mentioning. Earlier it was hypothesized that the occurance of pseudopregnancy leads to increased chances of canine breast tumour .Something which later was falsified. Last but not least its receptor was also put under the spotlight demonstrating prolactin importance as an upstream mediator in luteal steroid regulation .Pseudopregnancy was also observed after ovariectomy .So in this thesis several research investigations are analysed putting prolactin into the scope and checking its association to pseudopregnancy.

Acknowledgements

As this incredible journey, has come to an end I can't ignore the people that helped me make my lifelong dream of becoming a veterinarian **true**. I would like to thank my parents and especially my mother for all the support. My brother and roommate, who always gave me strength to continue to fight when I struggled and lost hope. My friends Babbak Sehat, Kosima Karnutsch, Stella Economou, Miltos Ioannou and Ron Korabelnic which made the whole journey sweeter and memorable. I would especially like to thank Babbak Sehat for being my library partner, it wouldn't have been the same without him. Last but not least, my partner Amr Baraka for always being there by my side.

Enormous gratitude to a special lady and doctor which guided me through the conduction of this thesis, Dr. Muller.

References

1. Allen, W., 1986: Pseudopregnancy in the bitch: the current view on aetiology and treatment.*J Small Animal Practice*, 27(7). p. 419-424.

2. Beijerink, N., Kooistra, H., Dieleman, S., Okkens, A., 2004: Serotonin antagonist-induced lowering of prolactin secretion does not affect the pattern of pulsatile secretion of follicle-stimulating hormone and luteinizing hormone in the bitch. *Reproduction*, 128(2). p. 181-188.

3. Ben-Jonathan, N., Hnasko, R., 2001: Dopamine as a Prolactin (PRL) Inhibitor. *Endocrine Reviews*, 22(6). p. 724-763.

4. Ben-Jonathan, N., LaPensee, C., LaPensee, E., 2008: What Can We Learn from Rodents about Prolactin in Humans? *Endocrine Reviews*, 29(1). p. 1-41.

5. Bertram, R., 2005:. A mathematical model for the mating-induced prolactin rhythm of female rats. *AJP: Endocrinology and Metabolism*, 290(3). p. 573-582.

6. Bethea, C., Neill, J., 1980: Lesions of the Suprachiasmatic Nuclei Abolish the Cervically Stimulated Prolactin Surges in the Rat. *Endocrinology*, 107(1). p. 1-5.

7. Brisken, C., Kaur, S., Chavarria, T., Binart, N., Sutherland, R., Weinberg, R., Kelly, P., Ormandy, C., 1999: Prolactin Controls Mammary Gland Development via Direct and Indirect Mechanisms. *Developmental Biology*, 210(1). p. 96-106.

8. Cafazzo, S., Bonanni, R., Valsecchi, P., Natoli, E., 2014:. Social Variables Affecting Mate Preferences, Copulation and Reproductive Outcome in a Pack of Free-Ranging Dogs. *PLoS ONE*, 9(6) p. e98594.

9. Chakraborty, P., 1987:. Reproductive hormone concentrations during estrus, pregnancy, and pseudopregnancy in the Labrador bitch. *Theriogenology*, 27(6). p. 827-840.

10. Concannon, P., 1977:. Changes in LH, Progesterone and Sexual Behavior Associated with Preovulatory Luteinization in the Bitch. *Biology of Reproduction*, 17(4). p. 604-613.

11. Concannon, P., 2011: Reproductive cycles of the domestic bitch. *Animal Reproduction Science*, 124(3-4). p. 200-210.

12. Cortese, L., Ouva, G., Verstegen, J., Ciaramella, P. and Persechino, A., 1997: Hyperprolactinaemia and galactorrhoea associated with primary hypothyroidism in a bitch. *Journal of Small Animal Practice*, 38(12). p..572-575.

13. Egli, M., 2005: Prolactin secretory rhythm of mated rats induced by a single injection of oxytocin. *AJP: Endocrinology and Metabolism*, 290(3). p. E566-E572.

Formatted: Space After: 1,2 line

14. Egli, M., Bertram, R., Sellix, M. and Freeman, M. 2004: Rhythmic Secretion of Prolactin in Rats: Action of Oxytocin Coordinated by Vasoactive Intestinal Polypeptide of Suprachiasmatic Nucleus Origin.*Endocrinology*, 145(7). p. 3386-3394.

15. Egli, M., Leeners, B. and Kruger, T. 2010: Prolactin secretion patterns: basic mechanisms and clinical implications for reproduction. *Reproduction*, 140(5). p. 643-654.

16. Freeman, M. and Neill, J. 1972: The Pattern of Prolactin Secretion during Pseudopregnancy in the Rat: A Daily Nocturnal Surge12. *Endocrinology*, 90(5). p. 1292-1294.

17. Gerres, S., Hoffmann, B., 1994: Investigations on the role of progesterone in the endocrine control of overt pseudopregnancy in the bitch: Application of an antigestagen and effects on corpus luteum function. *Animal Reproduction Science*, 35(3-4). p. 281-289.

18. Gobello, C., Colombani, M., Scaglia, H., de la Sota, R.L., Goya, R., 2001a: Heterogeneity of circulating prolactin in the bitch. *Reprod. Nutr. Dev*,41(6). p. 505-511.

19. Gobello, C., de la Sota, RL., Goya, R., 2001b: A Review of Canine Pseudocyesis. *Reproduction in Domestic Animals*, 36(6). p. 283-288.

20. Gobello, C., Baschar, H., Castex, G., de la Sota, RL., Goya, RG., 2001c: Dioestrous ovariectomy: a model to study the role of progesterone in the onset of canine pseudopregnancy. *J Reprod Fertil Suppl*, 57. p. 55-60.

21. Gobello, C., de la Sota, R. and Goya, R. 2001d: Study of the change of prolactin and progesterone during dopaminergic agonist treatments in pseudopregnant bitches. *Animal Reproduction Science*, 66(3-4). p. 257-267.

22. Harvey, M., Cauvin, A., Dale, M., Lindley, S. and Ballabio, R., 1997: Effect and mechanisms of the anti-prolactin drug cabergoline on pseudopregnancy in the bitch. *Journal of Small Animal Practice*, 38(8). p. 336-339

23. Hoffmann, B., Hoveler, R., Hasan, S. and Failing, K. 1992: Ovarian and pituitary function in dogs after hysterectomy. *Reproduction*, 96(2). p. 837-845.

24. Ignacak A, Kasztelnik M, Sliwa T,Korbut A,Rajda K,Guzik T.J., 2012: Prolactin not only lactotrophin a "new" view of the old hormone , *Journal of physiolofy and pharmacology* 2, 63, 5., p. 435-443

25. Jöchle, W., 1997: Prolactin in Canine and Feline Reproduction. *Reproduction in Domestic Animals*, 32(4). p. 183-193.

26. Kooistra, H., den Hertog, E., Okkens, A., Mol, J. and Rijnberk, A., 2000: Pulsatile secretion pattern of growth hormone during the luteal phase and mid-anoestrus in beagle bitches. *Reproduction*, 119(2), p. 217-222.

27. Kooistra, H. and Okkens, A., 2001: Secretion of Prolactin and Growth Hormone in Relation to Ovarian Activity in the Dog. *Reproduction in Domestic Animals*, 36(3-4). p. 115-119.

28. Kowalewski, M., Michel, E., Gram, A., Boos, A., Guscetti, F., Hoffmann, B., Aslan, S. and Reichler, I., 2011: Luteal and placental function in the bitch: spatio-temporal changes in prolactin receptor (PRLr) expression at dioestrus, pregnancy and normal and induced parturition. *Reproductive Biology and Endocrinology*, 9(1). p. 109.

29. Kowalewski, M., 2014: Luteal regression vs. prepartum luteolysis: Regulatory mechanisms governing canine corpus luteum function. *Reproductive Biology*, 14(2). p. 89-102.

30. Michel, E., Rohrer Bley, C., Kowalewski, M., Feldmann, S. and Reichler, I., 2012: Prolactin - to be reconsidered in canine mammary tumourigenesis?. *Veterinary and C`omparative Oncology*, 12(2). p. 93-105.

31. Niswender GD., Juengel, JL., Silva, PJ., Rollyson, MK., MCIntush, EW., 2000: Mechanisms Controlling the Function and Life Span of the Corpus Luteum. *Physiological reviews*, 80(1). p. 1-29.

32. Okkens, A., Dieleman, S., Bevers, M., Lubberink, A., Willemse, A., 1986: Influence of hypophysectomy on the lifespan of the corpus luteum in the cyclic dog. *Reproduction*, 77(1). p. 187-192.

33. Okkens, A., Dieleman, S., Bevers, M. and Willemse, A., 1985: Evidence for the non-involvement of the uterus in the lifespan of the corpus luteum in the cyclic dog. *Veterinary Quarterly*, 7(3). p. 169-173.

34. Papa, P., Hoffmann, B., 2011: The Corpus Luteum of the Dog: Source and Target of Steroid Hormones? .*Reproduction in Domestic Animals*, 46(4). p. 750-756.

35. Paul, M., Majumder, S., Bhadra, A., 2014: Grandmotherly care: a case study in Indian free-ranging dogs. *Journal of Ethology*, 32(2). p. 75-82.

36. Razzaque, WAA., Husain K., Agarwal S., Kumar, S., 2008: False pregnancy in bitch. *Veterinary World*, 1(3). p. 92-95.

37. Reppert, S., Weaver, D., 2002: Coordination of circadian timing in mammals. *Nature*, 418(6901). p. 935-941.

38. Sangeeta, DY., Halperin, J., 2014: Reproductive actions of prolactin mediated through short and long receptor isoforms. *Molecular and Cellular Endocrinology*, 382(1). p. 400-410.

39. Smith, M., Freeman, M., Neill, J., 1975: The Control of Progesterone Secretion During the Estrous Cycle and Early Pseudopregnancy in the Rat: Prolactin, Gonadotropin and Steroid Levels Associated with Rescue of the Corpus Luteum of Pseudopregnancy.. *Endocrinology*, 96(1). p. 219-226.

40. Smith, M., McDonald , L., 1974: Serum Levels of Luteinizing Hormone and Progesterone During the Estrous Cycle, Pseudopregnancy and Pregnancy in the Dog. *Endocrinology*, 94(2). p. 404-412.

41. Sorenmo, K., 2003: Canine Mammary Gland Tumors. *Veterinary Clinics of North America: Small Animal Practice*, 33, p. 573-596.

42. Toporikova, N., 2007: Regulation of Rhythmic Prolactin Secretion Combined Mathematical and Experimental Study. [Dissertation] URL:http://purl.flvc.org/fsu/fd/FSU_migr_etd-1277

43. Veronesi, M., Battocchio, M., Rizzi C., Sironi, G., 2003: Relationshio between Dysplastic and Neoplastic Mammary Lesions and Psedeupregnancy in the Bitch. *Veterinary Research Communications*, 27. p. 245-247.

44. Zubair, M., 2014: Causes of Infertility in Bitch. *Advances in Animal and Veterinary Sciences*, 2(10). p. 565-573.

I hereby confirm that I am familiar with the content of the thesis entitled
……...……...…….....
……..…...…….
written by ……..…….. (student name) which I deem suitable for submission and defence.

Date: Budapest, …….day……..month ……year
……

Supervisor's name and signature
……
……

Department

HuVetA – SZIA

ELECTRONIC LICENSE AGREEMENT AND COPYRIGHT DECLARATION*

Name:…….

Contact information (email):……..

Title of document (to be uploaded):…….
……..
……..

Publication data of document:….. ……..

Number of files submitted: ……..

By accepting the present agreement the author or copyright owner grants non-exclusive license to HuVetA and SZIA over the above mentioned document (including its abstract) to be converted to copy protected PDF format without changing its content, in order to archive, reproduce, and make accessible under the conditions specified below. The author agrees that HuVetA and SZIA may store more than one copy (accessible only to HuVetA and SZIA administrators) of the licensed document exclusively for purposes of secure storage and backup, if necessary. You state that the submission is your original work, and that you have the right to grant the rights contained in this license. You also state that your submission does not, to the best of your knowledge, infringe upon anyone"s copyright. If the document has parts which you are not the copyright owner of, you have to indicate that you have obtained unrestricted 4 permission from the copyright owner to grant the rights required by this Agreement, and that any such third-party owned material is clearly identified and acknowledged within the text of the licensed document.

The copyright owner defines the scope of access to the document stored in HuVetA/SZIA as follows (mark the appropriate box with an X):

☐ I grant unlimited online access,

☐ I grant access only through the intranet (IP range) of the Szent István University,

☐ I grant access only on one dedicated computer at the Veterinary Science Library,

☐ I grant unlimited online access only to the bibliographic data and abstract of the document.

_____

* The present declaration is based on Ordinance no. 5/2011 of the Rector concerning the scholarly database of institution (i.e. Szent Istvan University) affiliated publications.