Book Chapter

Role of FSH, LH and Prolactin in the Components of Litter Size in Female Rabbits

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Abstract

The aim of this study was to investigate the changes occurring in the luteinizing hormone (LH), the follicle-stimulating hormone

(FSH) and the prolactin (PRL) concentrations close to mating and whether the concentration of these hormones was related to components of litter size. Data from 60 primiparous females were used. Blood was taken 48 h before, and 2h and 48 h after the natural mating. Sera were analyzed by radioimmnunoassay for LH, FSH and PRL. A Laparoscopy was performed on all females at d 12 of the 2nd gestation. Ovulation rate (OR) and number of implanted embryos (IE) were recorded. The litter size (LS) at the 2nd birth was recorded. There were significative differences among LH concentration at 48 h before or after mating (5.1 and 5.4 ng/ml, respectively) *vs*. 2 h after mating (29.0 ng/ml). No differences in FSH and PRL were found in this period. No lactating females and females on d 18 of lactation showed higher LH and PRL concentrations than females on d 11 and 25 of lactation. All hormones were influenced by season, LH showed higher values in autumn than in summer, and on the contrary for PRL and FSH. The females with high OR (>15 ova), IE (>12 embryos) and LS (>8 kits) showed higher plasma LH concentration (15.1, 14.9, 15.5 ng/ml, respectively) than females with low OR, IE and LS (11.1, 9.6 and 9.7 ng/ml, respectively). The level of FSH influenced OR and LS, but only OR was affected by PRL. In conclusion, LH concentration, close to the mating, determined the imminent ovulation rate and the next implanted embryos and litter size. The level of FSH affected ovulation rate and litter size, and PRL concentrations only affected on ovulation rate.

Keywords

FSH; LH; PRL; Ovulation Rate; Implanted Embryos; Litter Size

Introduction

The reproductive hormone profiles in the rabbit does with different physiological status have been studied for a long time. For instance, the prolactin (PRL), the luteinizing hormone (LH), the follicle-stimulating hormone (FSH) or oestradiol 17-β have been referred in lactating females [1-3], in pregnant females [4,5], and at mating [5-7]. Also, the relationship between these hormones and the maternal nest-building behaviour has been

reported by Gonzalez-Mariscal *et al.* [8,9], Gonzalez-Mariscal [10], and Negatu and McNitt [11].

The components of litter size have been studied by different points of view, genetic [12] and phenotypic [13]. However, there is scarce bibliography about the relationship between the reproductive hormone profiles and the components of litter size, although, the ovulation rate and prenatal losses are a limiting factor of litter size in rabbits. Approximately, 30 to 40% of ova shed do not result in fetuses at term [14].

The aim of this study was to investigate the changes occurring in plasma LH, FSH and PRL concentrations 48h before the mating, 2 h and 48 h after the mating, as well as, the influence on ovulation rate, number of implanted embryos and litter size.

Materials and Methods

Animals and Experimental Design

A total of 60 primiparous females were used in this study. The does belong to a F2 population [15]. The rabbits were reared at the experimental farm of the Universidad Miguel Hernández de Elche, the does were caged individually with controlled light/dark cycle (16/8 h), and they had free access to a standard diet.

The first mating took place when the females were around 18 weeks old and, after delivering, the new mating was tried 11 days later. The reproductive rhythm was weekly, and the weaning was at 28 days of age. Any hormonal treatment was used in the experiment. Sexual receptivity was assessed based on the turgidity and colour of the vulva [16], and only receptive females were used in the experiment.

The blood samples were collected 48 h before mating, 2 h and 48 h after mating. A laparoscopy was performed on all females at day 12th of the 2nd pregnancy [12] and ovulation rate (OR), estimated as the number of corpora lutea, and number of implanted embryos (IE) were measured. Also, the litter size at 2nd birth was recorded (LS).

Blood Samples and Hormone Analyses

Blood samples were collected in heparinized tubes by puncture of ear vein. They were immediately centrifuged (5000 xg, 10 min, 4ºC) and plasma was stored at -20 ºC until assayed. Plasma concentrations of LH, FSH and PRL were determined in duplicate by RIA methods, using AFP-3120489, AFP-472176 and AFP-10304**,** antibodies for LH, FSH and PRL, respectively, provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and Dr. A.F. Parlow (Harbor UCLA Medical Center, CA, USA). The dilutions antibody used were 1: 1,500,000 for LH, 1:45,000 for FSH and 1:62500 for prolactin. For the iodination of the hormones was used the isotope ^{125}I, using the chloramine-T- method [17]. The volume of plasma used was 100 µl for LH, 75 µl for FSH, and 10 µl for PRL determinations. Staphylococcus aureus was used to precipitate the bound fraction [5]. The assay sensitivity were 0.05 ng/ml, 1 ng/ml and 0.04 ng/ml for LH, FSH and PRL, respectively, and the intra-assay coefficient of variation were <8% for all the hormones. The intra-assay coefficient of variation was estimated from plasma pool measured 8 times in the same assay.

Statistical Analysis

The LH, FSH and PRL concentration were analyzed by a model which included the fixed effect of time (48 h before mating, 2 h and 48 h after mating), the fixed effect of lactation (no lactating and females at 11, 18, 25 day of lactation), and the fixed effect of season (summer and autumn).

In order to analyze the influence of these hormonal levels on the components of litter size, the above model included the fixed effect of the ovulation rate (OR ≤ 15 ova; mean = 13.6 ova, and OR > 15 ova; mean = 18.3 ova), or the fixed effect of number of implanted embryos (IE ≤ 12 embryos; mean = 8.4 embryos, and IE > 12 embryos; mean = 14.9 embryos), or the fixed effect of the litter size at birth (LS ≤ 8 kits; mean = 5.1 kits, and LS > 8 kits; mean = 11.1 kits).

Only the significative interactions were included in the models. The GLM procedure of SAS (SAS Inst., Inc., Cary, NC) was used for these analyses.

Results and Discussion

Table 1 shows LH, FSH and PRL concentrations at 48 h before mating, 2 h and 48 h after mating. The LH concentration was the highest 2 h after mating (29.0 ng/ml), being 5 times higher than the LH concentration 48 hours before or after mating. This evolution of LH concentration was comparable with those observed by Ubilla *et al.* [2]. According to Rodriguez *et a*l. [6] LH level was 1.1 ng/ml after a GnRH challenge but LH increased fifteen min after GnRH administration between 200-1000% depending on the sexual receptivity of the does and the dose of GnRH.

The FSH and PRL concentrations were similar in the considered period. Ubilla *et al.* [3] also obtained no significant differences in FSH concentrations between 48 h before and 7 h after artificial insemination, and in PRL concentration between 48 h before and during the artificial insemination (Rebollar *et al.* 2000). In our experiment, the PRL level was, in general, lower than the data reported by the literature.

From our findings, there was not association of LH and FSH peaks and it agrees with results presented previously by Rodriguez *et al.* [18]. Thus, FSH concentrations could influence on the follicular growth, meanwhile LH concentrations could determinate the number of breakages of the different pre-ovulatory follicles.

No significant differences in LH concentration were observed among non-lactating females and on day 11, 18 and 25 of lactation. This result agrees with those previously studied by Ubilla *et al.* [2]. High FSH and PRL concentrations of non-lactating females and 18 days of lactation and low FSH and PRL concentrations at 11 and 24 day of lactation were found.

Table 1: Least square means (standard errors) of LH, FSH and PRL concentrations for time, lactation and season.

		LH (ng/ml)	FSH (ng/ml)	PRL (ng/ml)
Time, referred to the mating	- 48 h	5.1 (2.0) [a]	17.8 (1.84)	2.2 (0.17)
	+ 2 h	29.0 (2.1) [b]	16.1 (1.84)	2.1 (0.21)
	+ 48 h	5.4 (2.0) [a]	18.9 (1.85)	1.9 (0.21)
Lactation	No lactation	14.8 (1.59)	21.6 (1.36) [c]	2.3 (0.17) [bc]
	11 days	10.2 (2.51)	6.1 (2.65) [a]	1.7 (0.27) [ab]
	18 days	14.5 (3.55)	25.9 (3.70) [c]	2.7 (0.38) [c]
	24 days	13.0 (2.31)	16.8 (2.21) [b]	1.6 (0.25)
Season	Summer	8.6 (1.6) [a]	21.9 (1.91) [b]	2.3 (0.17) [b]
	Autumn	17.7 (1.9) [b]	13.3 (1.80) [a]	1.8 (0.20) [a]

[a,b,c] Means with different letters on the same column differ significantly ($P<0.05$).

The interaction between lactation and the season was only significative for FSH concentrations (Figure 1). In summer, the females on day 18 of lactation showed the highest FSH (40.6 ng/ml). However, in autumn the concentration was similar on day 11, 18, 25 of lactation and non-lactating females.

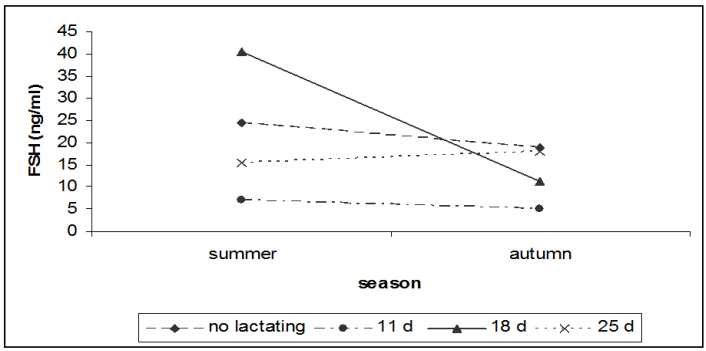

Figure 1: Interaction between lactation and season for FSH.

All the studied hormones were influenced by season, in summer the does showed lower LH than in autumn, but higher FSH and PRL (Table 1). The interaction between season and time was only significative for LH (Figure 2). In summer, the LH concentration showed 2.5-fold increase from 5.8 ng/ml to 14.7 ng/ml between 48 h before and 2 h after mating. In autumn LH

concentration showed 10-fold increase (4.3 to 43.2 ng/ml), although the components of litter size were similar in both seasons (15.7 ova, 12.1 implanted embryos and 8.3 kits in summer; 14.9 ova, 11.2 implanted embryos and 7.5 kits in autumn).

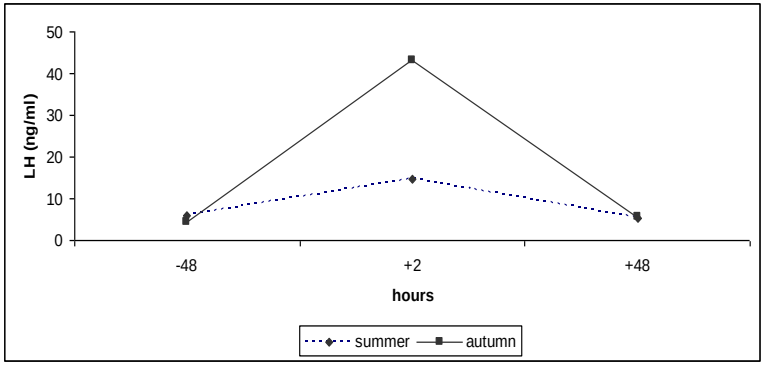

Figure 2: Interaction between season and time, referred to the mating, for LH.

Table 2 shows the influence of LH, FSH and PRL on components of litter size. The females with low OR (≤ 15 ova,) showed lower LH concentration than females with high ovulation rate (> 15 ova), in time close to the mating. In this period, the LH concentration also affected the IE at 12 days of gestation and LS, because females with low IE and LS showed lower LH level than females with high IE and LS (9.6 vs 14.9 ng/ml for IE; 9.7 and 15.5 ng/ml for LS). Figure 3 shows the significative interaction between the two levels of OR, IE and LS and the time. The LH concentration was around 6-fold higher 2h after mating in females with high OR (4.6, 35.6 and 5.6 ng/ml, -48h, 2 h and +48 h to the mating), but only 4-folder higher in females with low OR (5.5, 22.8 and 5.2 ng/ml, respectively). The females with more than 12 implanted embryos or more than 8 rabbits born also presented higher LH concentrations 2 h after the mating than females with low than 13 implanted embryos and 9 rabbits born.

Table 2: Least square means (standard errors) of LH, FSH and PRL concentration for ovulation rate (OR), implanted embryos (IE) and litter size (LS).

		LH (ng/ml)	FSH (ng/ml)	PRL (ng/ml)
OR	≤ 15	11.1 (1.60) [a]	14.1 (1.49) [a]	1.7 (0.17) [a]
	>15	15.1 (1.80) [b]	21.1 (1.82) [b]	2.4 (0.18) [b]
IE	≤ 12	9.6 (2.16) [a]	16.1 (1.85)	1.9 (0.21)
	>12	14.9 (1.76) [b]	17.1 (1.63)	2.0 (0.17)
LS	≤ 8	9.7 (1.94) [a]	13.6 (1.58) [a]	1.9 (0.19)
	>8	15.5 (1.79) [b]	15.4 (1.45) [b]	2.1 (0.18)

[a,b] Means with different letters on the same column differ significantly ($P<0.05$).

The FSH concentrations were different between females with lower OR and LS (14.1 and 13.6 ng/ml) than females with high OR and LS (21.1 and 15.4 ng/ml). The PRL concentration only affected OR. The females with OR lower o equal to 15 ova had lower PRL concentration than females with OR higher than 15 ova. Fortun *et al*. [19] found that fetal mortality was increased by PRL treatment during gestation, but in our experiment the PRL concentration, at the time close to the mating, is similar for females with different number of IE.

Conclusions

Although FSH and LH are both hypophysarian hormones and stimulate the ovarian activity, the LH concentration, close to the mating, determines the imminent ovulation rate and the next implanted embryos and litter size. The level of FSH has influenced on the ovulation rate and litter size, and PRL concentrations only affected on ovulation rate.

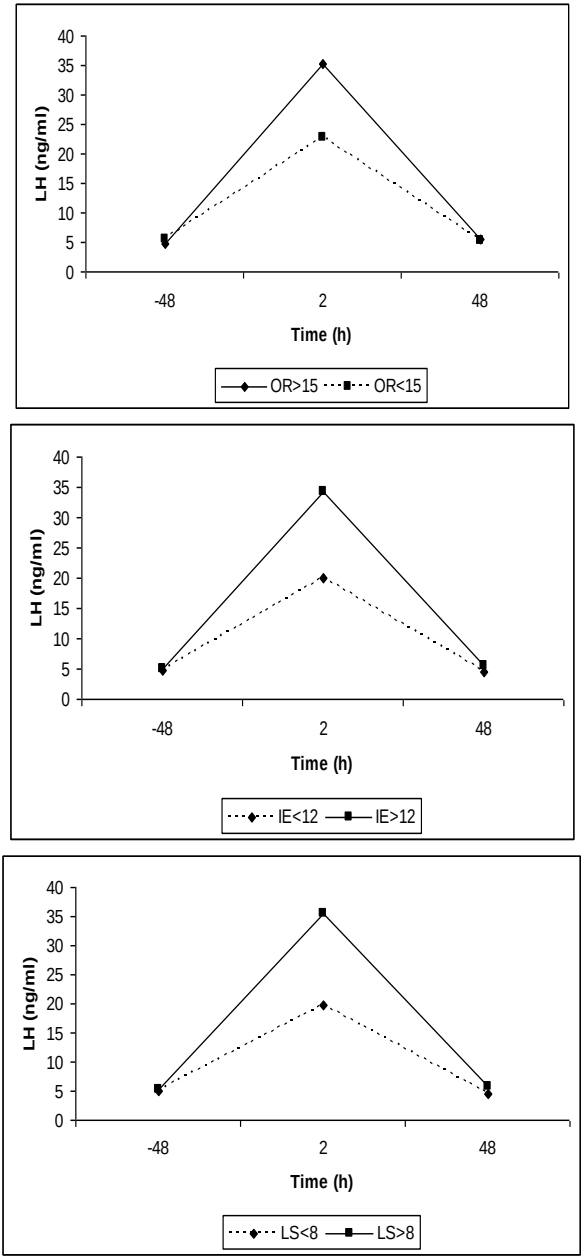

Figure 3: Interaction between ovulation rate (OR), implanted embryos (IE) and litter size (LS) with the time referred to the mating, for LH.

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