INFLUENCE OF FSH, LH AND PROLACTIN ON THE COMPONENTS OF LITTER SIZE IN RABBIT DOES

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ABSTRACT

The aim of this study was to investigate whether the concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) around mating were related to the components of litter size. Data from 60 primiparous females were used. Blood was taken 48 h before, and 2 and 48 h after the natural mating. Sera were analyzed by radioimmnunoassay for LH, FSH, and PRL. Laparoscopy was performed at day 12 of gestation to record ovulation rate (OR) and number of implanted embryos (IE). The litter size (LS) at birth was recorded. There were significant differences among LH concentration measured 48 h before or after mating (5.1 and 5.4 ng/ml, respectively) vs. 2 h after mating (29.0 ng/ml), but not for FSH and PRL. Non lactating females and females at day 18 of lactation showed higher LH and PRL concentrations than females on days 11 and 25 of lactation. All the hormones were influenced by the season, LH showing higher values in autumn than in summer, in opposition to both 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 PRL affected only OR.

Key words: FSH, LH, PRL, Ovulation rate, Embryos.

INTRODUCTION

The reproductive hormone profiles of rabbit does at different physiological status have been studied for a long time. For instance, PRL, LH, FSH, and oestradiol-17β were investigated in lactating females (Lamb *et al.*,1993; Ubilla *et al*., 2000a, 2000b), in pregnant females (Challis *et al.*, 1973; Ubilla *et al.*,1992), and at mating (Rodriguez *et al.*, 1989; Ubilla *et al.*, 1992, 2000a, 2000b). The relationship between these hormones and the maternal nest-building behaviour has been reported by Gonzalez-Mariscal *et al.* (1996, 2000), Gonzalez-Mariscal (2001), and Negatu and McNitt (2002). The components of litter size have been studied by genetic (Argente *et al.*, 1997) and phenotypic (Santacreu *et al.*, 1992) points of view. However, there is scarce bibliography about the relationship between reproductive hormones 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 foetuses at term (Blasco *et al*., 1993). This study was designed to investigate whether the changes occurring in plasma LH, FSH, and PRL concentrations 48 before and 2 and 48 h after the mating, may explain ovulation rate, number of implanted embryos and litter size.

MATERIALS AND METHODS

Animals and experimental design

Sixty primiparous females, belonging to a F2 population (Peiró *et al*., 2007), reared at the experimental farm of the Universidad Miguel Hernández de Elche, were used in this study. The rabbits, aged 17 weeks, were caged individually with controlled light/dark cycle (16/8 h), and had free

access to a standard diet. The does were mated 11 days after parturition, with a weekly rhythm; weaning was at 28 days of age. No hormonal treatment was used in the experiment. Only sexual receptive females, assessed on the basis of vulva turgidity and colour (McNitt and Moody, 1989), were used in the experiment. Ovulation rate (OR), defined as number of corpora lutea, and number of implanted embryos (IE) were estimated by laparoscopy, performed on all the females at the 12th day of pregnancy (Santacreu *et al.*, 1990), while litter size (LS) was recorded at birth.

Blood samples and hormone analyses

Blood samples were taken in females lactating (11, 18, 25 after parturition) and in non lactating females (32 day after parturition) and were obtained by puncture of the ear vein 48 h before mating and both 2 and 48 thereafter. The samples, collected in heparinized tubes were immediately centrifuged (5000 x g, 10 min, 4ºC) and plasma was stored at -20ºC until assayed. Plasma concentrations of LH, FSH, and PRL were determined in duplicate by RIA, 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 by Dr. A.F. Parlow (Harbor UCLA Medical Center, CA, USA). The dilutions antibody used were 1:1,500,000, 1:45,000 and 1:62500 for LH, FSH, and PRL. Hormones were iodinated with ^{125}I, using the chloramine-T- method (Green-wood *et al.*, 1963). The volumes of plasma were 100, 75, and 10 µl for LH, FSH, and PRL determinations, respectively. Staphylococcus aureus, prepared by the Autónoma University, Departament of Plant Physiology (UAM, Madrid), was used to precipitate the bound fraction (Ubilla *et al.*, 1992). The assay sensitivity were 0.05, 1.0 and 0.04 ng/ml for LH, FSH, and PRL, respectively; the intra-assay coefficients of variation, estimated from plasma pool measured 8 times in the same assay, were <8% for all the hormones.

Statistical Analysis

The plasma LH, FSH and PRL concentration were analyzed by a model which included the fixed effect of time (with 3 levels: 48 h before the mating, 2 h and 48 h after the mating), the fixed effect of stage of lactation (with 4 levels: non lactating females and females at 11, 18, 25 days of lactation, referred at the mating), and the fixed effect of season (with 2 levels: summer and autumn). To analyze the influence of hormonal concentrations on the components of litter size, the model included the fixed effect of the ovulation rate (with 2 levels: OR≤15 (mean=13.6) and OR>15 (mean=18.3)), or the fixed effect of number of implanted embryos (with 2 levels: IE≤12 (mean=8.4) and IE>12 (mean=14.9)), or the fixed effect of the litter size at birth (with 2 levels: LS≤8 (mean=5.1) and LS>8 (mean=11.1)). Only the significant interactions were included in the models. The GLM procedure of SAS (SAS Inst., Inc., Cary, NC) was used for these analyses.

RESULTS AND DISCUSSION

There were significant differences among LH plasma concentrations (Table 1) measured 48 h before or after the mating (5.1 and 5.4 ng/ml, respectively) vs. 2 h after the mating (29.0 ng/ml). This pattern was comparable with that observed by Ubilla *et al.* (2000a). According to Rodriguez *et al*. (1989), LH was 1.1 ng/ml before GnRH challenge, but increased 200-1000% 15 min later depending on sexual receptivity of does and GnRH dose.

The FSH and PRL concentrations did not change in the time frame here considered. Ubilla *et al.* (2000b) did not find any difference in FSH concentrations between 48 h before and 7 h after artificial insemination. Similarly, Rebollar *et al*. (2000) did not observe any change in PRL concentrations between 48 h before and during the artificial insemination. In our experiment, the PRL levels were, in general, lower than those reported in the literature.

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

		LH (ng/ml)	FSH (ng/ml)	PRL (ng/ml)
Time, referred to 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
Stage of 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 [a b]
	18 days	14.5 ± 3.55	25.9 ± 3.70 [c]	2.7 ± 0.38 [c]
	25 days	13.0 ± 2.31	16.8 ± 2.21 [b]	1.6 ± 0.25 [a]
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]

Means with different letters on the same column differ significantly (P<0.05)

From our findings, there was no association between LH and FSH peaks in agreement with previous results reported by Rodriguez *et al.* (1989). So, FSH may influence the follicular growth, meanwhile LH may determine the number of breakage of pre-ovulatory follicles. No differences in LH concentration were observed among non lactating females and at days 11, 18, and 25 of lactation (Table 1). These results are in agreement with those previously described by Ubilla *et al.* (2000a). High FSH and PRL levels on non lactating females and at 18 days of lactation and low FSH and PRL levels on 11 and 25 day of lactation were found (Table 1).

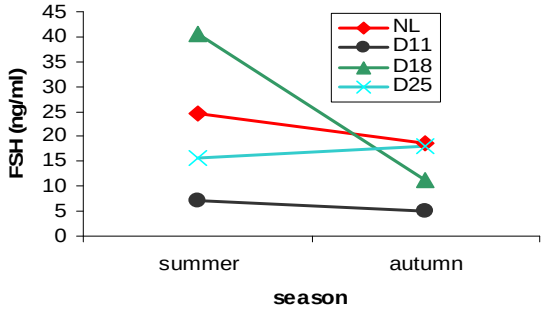

Figure 1: Interaction between stage of lactation (non lactating (NL), and at 11 (D11), 18 (D18), 25 (D25) days of lactation) and season for FSH

The interaction between stage of lactation and season was significant only for FSH (Figure 1). In summer, the females at day 18 of lactation showed the highest FSH (40.6 ng/ml). However in autumn, the concentrations were similar at days 11, 18, and 25 of lactation and in non lactating females. All the hormones were influenced by the season; in summer the does showed lower LH than in autumn, but higher FSH and PRL (Table 1). The interaction between season and time relative to mating was only significant for LH (Figure 2). In summer, LH levels showed 2.5-fold increase, from 5.8 to 14.7 ng/ml, between 48 h before and 2 h after the mating. In autumn, LH concentrations showed a 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).

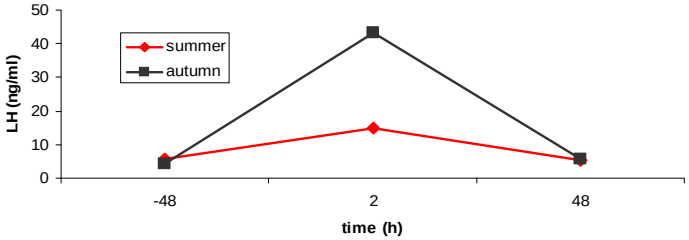

Figure 2: Interaction between season (summer and autumn) and time, referred to the mating, for LH

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 level than females with high OR (>15 ova), around mating. In this period, the LH level also affected 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 vs. 15.5 ng/ml for LS). Figure 3 shows the interaction between the two levels of OR, IE and LS and the time. The LH levels were 6-fold higher 2 h after the mating in does with high OR (4.6, 35.6 and 5.6 ng/ml, -48, 2 and +48 h from mating, respectively), 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 IE and low LS.

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

		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

Means with different letters on the same column differ significantly (P<0.05)

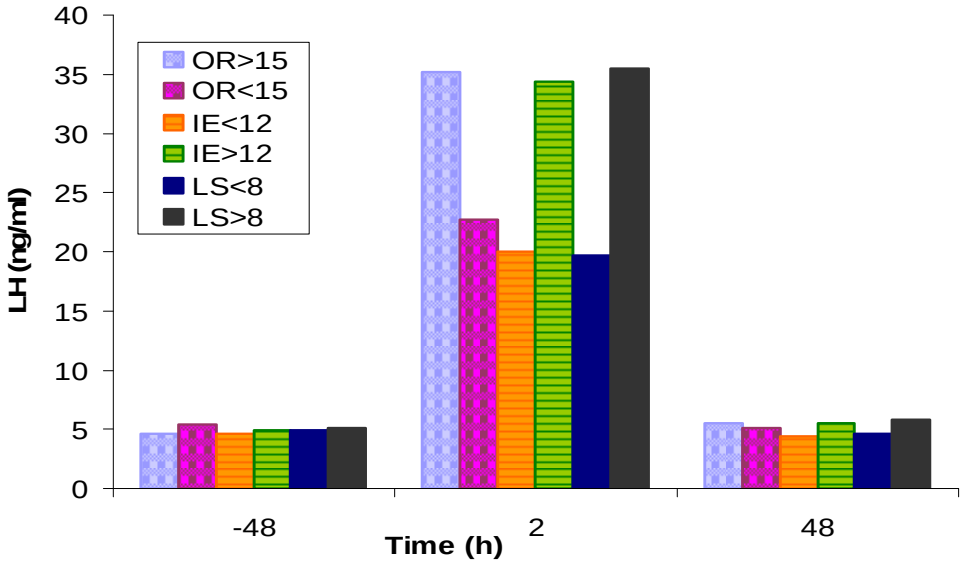

Figure 3: Interactions between ovulation rate (OR), implanted embryos (IE), and litter size (LS) with the time, referred to the mating, for LH

The FSH levels 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. Fortun *et al*. (1994) found that foetal 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 stimulating the ovarian activity, the plasma LH concentration close to the mating determines the imminent ovulation rate as well as the number of next implanted embryos and litter size. The level of FSH influences ovulation rate and litter size, while PRL concentrations only affects ovulation rate.

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