EFFECT OF POSTPARTUM PROGESTERONE LEVELS ON RECEPTIVITY, OVARIAN RESPONSE, EMBRYO QUALITY AND DEVELOPMENT IN RABBITS

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Abstract - Peripheral plasma progesterone (P) has been evaluated in 245 multiparous (M) and 91 nulliparous (N) hybrid female rabbits in 3 trials. Blood samples were collected from each animal immediately prior of artificial insemination and 3 d before. The rabbits were inseminated 11 d postpartum (130 d of age for the N). Ovulation was induced by 0.8 µg of GnRH. On the average, 21.1% (range 4.6% to 34.3% among rabbitries) of M does and only 3.3% of N females showed high P levels (≥ 2.0 ng/ml). Moreover, blood basal progesterone levels where higher in M P- does than in N P- rabbits (0.62±0.28 vs 0.14±0.16 ng/ml, respectively; P<0.01). Among P- does, 56% were sexually receptive against 11.5% of the P+. High levels of progesterone significantly reduced the embryo recovery rate (0.4 vs 0.9 for P+ and P- does, respectively) and embryo quality. In addition the pregnancy rate was significantly lower in P+ than in P- (12.9% vs. 66.4%; P<0.01).

INTRODUCTION

In the rabbit ovary, progesterone exerts an inhibitory action on both follicular development and steroidogenic function (SETTY and MILLS, 1987). This action appears to be exerted directly via an intraovarian route, rather than indirectly by way of suppression of gonadotropin secretion (MILLS and STOPPER, 1989). Progesterone also has been shown to inhibit sexual receptivity in ovariectomized rabbits primed with oestrogens (BEYER and MCDONALD, 1973). In the rabbit, elevated progesterone levels at the time of mating or artificial insemination have been associated with impaired fertility characterized by delayed ovulation (KENELLY and FOOTE, 1965), altered gamete transport (TAKEDA *et al.*, 1978) and a high incidence of degenerated embryos (TSUTSUMI *et al.*, 1980). Since we sometimes found abnormally high progesterone concentrations in lactating does between day 4 and 15 postpartum (data not published), the aims of this study were 1) to assess the progesterone concentrations in the early postpartum period in different commercial rabbitries, 2) to correlate high (P+) and low (P-) progesterone levels with sexual receptivity, and finally 3) to evaluate the influence of progesterone on ovarian response, embryo recovery rate and *in vitro* embryo development in artificially inseminated rabbit.

MATERIALS AND METHODS

Animals and treatments

For this study 91 nulliparous and 245 multiparous (1-4 kindling order) hybrids female rabbits homogeneous for body weight and litter size (6-8 newborn) were used. The animals were individually caged and fed *ad libitum* with a commercial pelleted diet. Controlled environmental conditions of temperature (22-25°C) and light cycles (14L:10D) were assured. The rabbits in experiments 2 and 3 were artificially inseminated (a.i.) at 11 days postpartum with fresh semen collected from bucks of proven fertility while the nulliparous at 130 days of age. Ovulation was induced by i.m injection of 0.8 µg of GnRH (Receptal, HOECHST).

Experiment 1- The experiment was pointed to evaluate progesterone concentrations in the postpartum period and therefore blood samples were collected from 22 primiparous does every other day from day 2 after parturition until day 18. Between day 16 and 18, 3 P+ and 3 P- does were sacrificed for morphological evaluation of ovarian structures.

Experiment 2 - The experiment was carried out on two homogeneous groups of 91 nulliparous and 108 multiparous hybrids from two different rabbitries in order to evaluate the correlation between progesterone concentrations and fertility. Blood samples were obtained from each animal immediately prior of a.i. and 3 days before.

Experiment 3 - This study was specifically devised to evaluate the effect of naturally high or low progesterone levels on sexual receptivity, ovarian response, embryo quality and development *in vitro*. For this purpose 115 multiparous lactating does were used. Blood samples were collected as described in experiment 2. Sexual receptivity, scored on the basis of the vulva colour from pale (R-) to red and purple associated with turgescence (R+), was estimated by experienced technicians. Forty-eight hours post a-i. 38 does were sacrificed and for each ovary number of fresh corpora lutea (CL) induced by GnRH administration and postpartum corpora lutea (CLp) was recorded. Ovulatory response defined as percentage of does with at least 1 CL, and ovulatory rate as mean number of CL per animal which ovulated were also determined. Recovered embryos were morphologically evaluated for stage of development and assigned to a quality grade score (A=excellent, B=good, C=fair, D=degenerate) as outlined by LINDNER and WRIGHT (1983). Embryo recovery rate (number of recovered embryos/number of CL) were calculated and embryo development rate was then assessed after 72 hours of *in vitro* culture as previously reported by STRADAIOLI *et al.* (1993).

Progesterone assay

Blood samples were collected into heparinized vacutainers by venopuncture from the marginal ear vein of each animal and plasma was separated by centrifugation and stored at -20°C until assay. Plasma progesterone concentrations were evaluated by a specific and standardized RIA procedure (BOITI *et al.* 1974). The sensitivity of the assay for a sample volume of 200 µl was 0.08 ng/ ml. The intra and interassay variation coefficients were 5.3% (n=8) and 10.2% (n=4), respectively. In order to discriminate the P+ from the P- female rabbits a value of 2.0 ng/ml was arbitrarelly set.

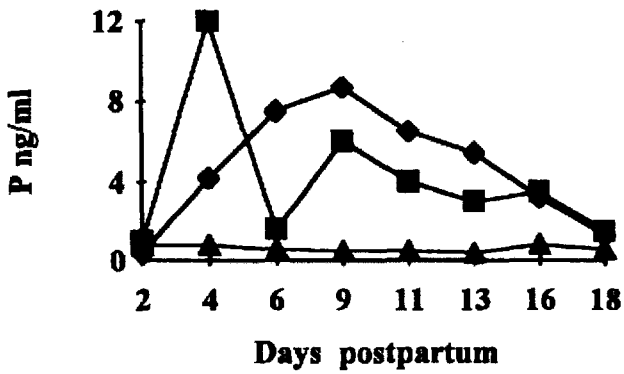
Statistical analysis

Data were analyzed using GLM and FREQ procedures (SAS, 1991).

RESULTS AND DISCUSSION

Progesterone levels in the postpartum

Figure 1 : Examples of progesterone (P) profiles in early postpartum period



▲ profile 1 ; ◆ profile 2 ; ■ profile 3

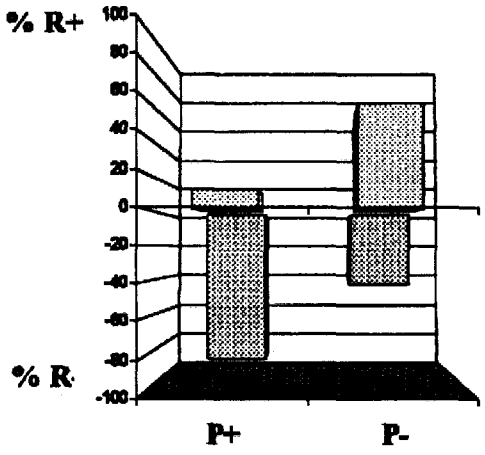
Examples of three peripheral plasma progesterone profiles during the postpartum of primiparous does (Experiment 1) are depicted in Figure 1.

Progesterone concentrations were already at basal levels 2 days after parturition in 70% of the does (n=16) and fluctuated between 0.4 and 0.6 ng/ml during the following 3 weeks (profile 1). In the other 30% of the females (n=6), blood progesterone was very high, exceeding 5 ng/ml, on day 4 postpartum with a variable profile thereafter. In two of these lactating does the period of luteal progesterone secretion extended for 13-15 days (profile 2), comparable to the functional lifespan of CL of pseudopregancy (GADSBY, 1989) while in the other 4 subjects, progesterone had peaks of shorter duration (profile 3). The 3 P+ does that were sacrificed showed enlarged ovaries and an average of 2.6 CLp per ovary, while the 3 P- had only few

corpora albicantia.
It is well established that during both pregnancy and pseudopregnancy circulating progesterone concentrations reflect the rate of its synthesis and secretion by the corpus luteum which is essentially the major source of progesterone in the rabbit (HOLT, 1989). According to MILLS and STOPPER (1989) plasma progesterone is also closely related to the number of CL: in rabbits with single CL, its concentrations are well below those found in rabbits with two or more CL (1.8±0.3 vs 7.0±0.8 ng/ml). Interstitial ovarian cells are the primary source of 20α-dihydroprogesterone, but could also secrete progesterone at basal levels (BREED and HILLIARD, 1970). The low progesterone profiles we found in the early postpartum period in 16 out of 22 does are in agreement with the observation of BREED and HILLIARD (1970) who reported that the CL regress quickly within 3 days after parturition. The finding of variably high progesterone profiles during early postpartum in 6 of 22 females suggests that spontaneous postpartum ovulations could occur in the rabbit does, as already observed by MYERS and POOLE (1962), following male sexual advances. It should not be excluded that repeated manipulations, due to frequent blood samplings, triggered ovulation in particularly sensitive subjects.
The multiparous P+ females (Experiments 2 and 3) averaged 17,I% (n=38) at day 8 postpartum and rose to 21,1% (n=47) at day 11 ; this percentage varied significantly (P<0.01) among rabbitries, ranging from 4.6% to 34.3%. By contrast, only 3.3% of the nulliparous (n=3) were P+. Furthermore, when compared with the nulliparous, the multiparous P- does were characterized by significantly higher blood basal progesterone levels (0.62±0.28 vs. 0,14±0,16 ng/ml; P<0.01). Mean progesterone concentrations of the multiparous P+ does were no different between day 8 and day 11 (6,1±3.58 vs 6.8±3.41 ng/ml), but were higher than those found in nulliparous P+(2.5±0.6 vs. 2.3±0.7 ng/ml; n= 3), although not significantly probably due to the low number of the later samples. Even if the two blood samples, which were taken on day 8 and 11 after parturition are clearly not sufficient to elucidate progesterone profiles during the postpartum period, they allow us to reliably identify P+ and P- rabbit does. In addition, this procedure drastically reduces possible effects due to repeated manipulations.

Progesterone levels and sexual receptivity.

Figure 2 : Sexual receptivity (R) as related to progesterone levels (P)



On the day of a.i. only 46.2% of the does (53/115) were receptive (R+) according to our classification based on vulval coloration. This procedure is not as accurate as direct observation of sexual behaviour of lordosis in the presence of the male, but is much more practical to perform (MAERTENS and LUZI, 1995). Among the 89 P- does (Figure 2) about half were also R+ (56%). By contrast, within the 26 P+ females proportion of oestrous does (R+) was significantly low (11.5%= P<0.01).
Sexual receptivity plays an important role in the reproduction of domestic rabbits, also in the case of a.i. when does are inseminated independently of their oestrous behaviour (THEAU-CLEMENT and ROUSTAN, 1992). Sexual receptivity varies during postpartum (MORET, 1980) with irregularly alternating periods of oestrous and anoestrous. While progesterone is essential for embryo survival (FORTUN *et al.*, 1993), this hormone is not necessary for the expression of sexual behaviour in the rabbit (FRENCH, 1977; ELSAESSER, 1980). Moreover, the observation that in both pregnant and pseudopregnant rabbits sporadic sexual receptivity signs occur, although in an irregular manner, suggests that progesterone is the major hormonal factor which inhibits sexual behaviour. In ovariectomized rabbits (BEYER and MCDONALD, 1973) oestradiol induces sexual behaviour, whereas progesterone inhibits it. Using active immunization techniques, ELSAESSER (1980) showed that oestrogens were necessary for receptivity while progesterone inactivation did not interfere with it. In this specie, receptivity is dependent upon the presence of ovarian steroids and particularly oestrogens, also if peaks of oestrone or oestradiol-17β do not always coincide with oestrous behaviour (CAILLOL *et al.*, 1983). Receptive does have been found to have a higher number of large follicles than non receptive females

(KERNABON *et al.*, 1994) and also increased oestrogen levels (UBILLA and REBOLLAR, 1995). In our study no attempt was made to determine circulating oestrogen because their levels vary widely, also within the same female, and therefore much more frequent blood sampling would be required for an accurate correlation between these sex steroid hormonal levels and receptivity.

Progesterone and fertility

The pregnancy rate of the multiparous does (Experiment 2) was strongly affected by progesterone levels (66.4% vs 12.9% in P- and P+ respectively; P<0.01) whereas litter size was not influenced (8.3 vs 7.9 respectively). No differences between pregnancy rate and litter size were observed in P+ and P- nulliparous (average 80. 7% and 6.3), probably due to the low number of females with high progesterone concentrations.

Progesterone, ovarian response and in vitro embryo development

Data concerning the ovarian response and *in vitro* embryo development in the P+ (6.4±4.1 ng/ml, n=7) and P- (0.8±0.2 ng/ml, n=31) does sacrificed 48 hours post-insemination are shown in Table 1. Moreover, the presence of CLp in 6 out of 7 P+ females confirms that spontaneous ovulations may occur during early postpartum in a significant percentage of the rabbit female population before mating or artificial insemination. Ovulation induced by GnRH was not influenced by high blood progesterone levels as all the P+ females responded and presented an ovulatory index comparable to that of the P- does (Table 1). However, high levels of progesterone significantly reduced the embryo recovery rate and quality, probably acting on oocyte maturation, gamete transport and fertilization. We found no influence of progesterone level on *in vitro* development of good quality embryos.

Table 1 : Effect of progesterone class (P+, P-) on the reproductive characteristics of does

Items		P+	P-	Signif.
Number of does	n.	7	3 1	
Ovulatory response	%	loo	96.3	n.s.
Ovulatory rate	n.	13.5	1 1.2	n. s
Embryo recovery rate		0.4	0.9	**
Embryo (quality A-C)	n.	0.6	8.7	**
Embryo (quality D)	n.	4.3	1.0	**
Embryo development 72 h	%	loo	95.4	n.s.

**: P < 0.01; n.s.: not significant.

In conclusion, our study demonstrated the presence of a high percentage of P+ animals in field condition, especially in multiparous does (21,1%), while it was occasionally observed among nulliparous females (3.3%). These high levels of progesterone are associated with reduced sexual receptivity, low pregnancy rate, low embryo recovery rate and embryo quality. It remains to be established which factors could trigger spontaneous ovulation, as indicated by the presence of CL, high blood progesterone concentrations and its secretory patterns in the postpartum period. Furthermore, a high variability among rabbitries and parity was observed. Our data are not in agreement with those reported by LAMB *et al.* (1991) who found relatively low progesterone concentration on day 1 and 14 postpartum in primiparous crossbreed does, but this discrepancy could be due in large part to the different strains of rabbits used and rearing management techniques.

There is no doubt that complex interactions among lactation, receptivity and ovarian competence modulate the fertility of the female rabbit during the early post-partum period. However, the finding of abnormally high progesterone levels on the day of artificial insemination in a significant proportion of multiparous does under field condition could in part explain their reduced reproductive performance. It could also open new questions about the appropriateness of hormonal treatments, presently employed under intensive breeding programs to induce oestrous synchronization, which are not tailored to their endocrinological status.

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Effets du taux de progesterone postpartum sur la réceptivité, la réponse ovarienne et la qualité embryonnaire chez la lapine - On a mesuré le taux de progesterone (P) dans le plasma périphéfique chez 245 lapines hybrides multipares (M) et 91 nullipares (N) au cours de 3 expérimentations. Les échantillons de sang ont été recueillis 3 jours avant et immédiatement avant l'insémination artificielle. Les lapines ayant été inséminées au 11ème jour postpartum (et à l'age de 130 jours pour les N). L' ovulation a été provoquée par 0.8 µg de GnRH. En moyenne, 21,1% des femelles M (de 4.6% à 34.3% parmi les différents élevages) et 3.3% des N ont montré des taux élevés de P (≥2.0 ng/ml). De plus, les taux minimum de progesterone dons le sang étaient plus élevés chez les M que chez les N (0. 62±0.28 contre 0,14±0.16 ng/ml respectivement; P<0.01). Parmi les femelles ayant un taux réduit de progesterone (P-) 56% étaient sexuellement réceptives contre 11.5% de celles présentant un taux élevé (P+). Des taux élevés de progesterone réduisent de manière significative le taux de récupération embryonnaire (0.4 contre 0.9 pour P+ et P- respectivement) ainsi que la qualité des embryons. De plus, le taux de fécondité était significativement plus bas pour les P+ que pour les P- (12.9% contre 66.4% P<0.01).