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Original article

Influence of progesterone supplementation on foetal survival in concurrently pregnant and lactating does

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Summary — An experiment was conducted to assess the influence of progesterone levels on late foetal mortality (second half of gestation) in concurrently pregnant and lactating does. All the females were mated within 12 h of parturition (d 0), and slaughtered on d 28 to study reproductive performance and body composition. Females were allowed to lactate 10 young and received either 2 progesterone implants (group P, $n = 29$) or 2 empty implants (group C, $n = 29$) on d 7. The feed intake, the live-weight variations of females during gestation, the growth of suckling litters, as well as the foetal weight and body composition of the does at slaughter, were similar in both groups. The concentration of progesterone was higher in group P than in C on d 16 and 25 ($P < 0.01$). The ovulation rate and late foetal mortality were similar in both groups but the early mortality was lower (12.3 *vs* 21.2%; $P < 0.05$) and the total number of foetuses on d 28 (live + resorbed + dead) was higher (9.8 *vs* 8.7; $P < 0.05$) in group P. These results indicate that progesterone supplementation improves early foetal survival but cannot increase late foetal survival in concurrently pregnant and lactating does.

rabbit / lactation / gestation / foetal death / progesterone

Résumé — Rôle de la progestérone sur la viabilité fœtale chez les lapines simultanément gravides et allaitantes. *L'objectif de cette expérience est de connaître l'effet d'une supplémentation en progestérone sur la mortalité fœtale tardive (2e moitié de la gestation) chez les lapines simultanément gravides et allaitantes. Toutes les femelles ont été saillies dans les 12 h suivant la parturition (j0) et abattues à j28 pour étudier les performances de reproduction et la composition corporelle. Les portées ont été égalisées à 10 lapereaux le jour de la saillie, et les femelles ont reçu 2 implants de progestérone (lot P, n = 29) ou 2 implants vides (lot C, n = 29) à j7. La consommation alimentaire ainsi que le poids vif des femelles et des portées ont été mesurés chaque semaine. Des prises de sang ont été effectuées à j7, 16 et 25. La consommation alimentaire, l'évolution du poids des femelles et des lapereaux sous la mère, de même que la composition corporelle des lapines et le poids des fœtus à l'abattage sont similaires dans les deux lots. La concentration en progestérone est supérieure dans le lot P à j16 et j25 (P < 0,01). Le taux d'ovulation et la mortalité fœtale tardive sont similaires dans les 2 lots, mais la mortalité précoce est inférieure (12,3% vs 21,2% ; P < 0,05) et le nombre total de fœtus à j28 est supérieur (9,8 vs 8,7 ;*

P < 0,05) chez les animaux du lot P. En conclusion, une supplémentation par implants de progestérone des lapines allaitantes augmente le taux de viabilité fœtale précoce, mais n'accroît pas le taux de viabilité fœtale tardive.

lapin / lactation / gestation / mortalité fœtale / progestérone

INTRODUCTION

Female rabbits are able to sustain concurrent pregnancy and lactation, but late foetal mortality (second half of pregnancy) is increased and foetal growth is decreased in lactating does compared with non-lactating does (Fortun *et al,* 1993).

Progesterone, entirely produced by the corpus luteum, is required to maintain pregnancy in rabbits (Thau and Lanman, 1974; Holt, 1989). Progesterone begins to increase on d 2–3, increases steadily until d 12–14 and drops sharply on d 28–29 before parturient activity (Challis *et al,* 1973; Thau and Lanman, 1975; Zoldag *et al,* 1988). The concentration of progesterone has been found to be significantly lower in concurrently pregnant and lactating does than in non-lactating ones (*eg* 11 ng/ml vs 15 ng/ml on d 17 of pregnancy; Fortun *et al,* 1993). Moreover, higher foetal mortality and lower progesterone concentrations were simultaneously observed in pregnant does treated with prolactin (Fortun, 1993). Thus, an effect of the lower progesterone concentration in the high foetal mortality observed in simultaneously pregnant and lactating does may be hypothesized.

To assess the role of progesterone levels in impaired foetal survival observed in concurrently pregnant and lactating does, reproductive performances were compared in lactating does, some of which were supplemented with this hormone, some of which were not.

The preovulatory administration of a low dose of progesterone induces asynchrony between the uterus and the embryos, and lowers the implantation rate (Schacht and Foote, 1978). Moreover, in rats, progesterone treatment during the first week of lactation affects lactational performance (Herrenkohl, 1972). However, progesterone does not block milk synthesis if lactation is well established (Herrenkohl, 1972). For these reasons, the progesterone implants were inserted on d 7, around the implantation of the embryos (Torrès, 1982), during established lactation.

In lactating does, impaired reproductive performance are associated with lower weights of carcasses, skin and adipose tissues of dams (Fortun *et al,* 1993). Moreover, progesterone supplementation can increase feed intake and the proportion of total body fat (Shirling *et al,* 1981; 1983). Therefore, body composition of the does was also determined at the end of pregnancy.

MATERIALS AND METHODS

Animals

Seventy primiparous crossbred does (22 weeks old) between A2066 and A1077 INRA lines were mated within 12 h of parturition (d 0) using 20 males from INRA line A1077. Fifty-eight females became pregnant. After mating, the females were allocated to experimental groups according to their litter size and body weight. Within 24 h of birth, litters were equalized at 10 young by cross-fostering and culling. The mean litter size before equalization was 10.1 young born alive. On d 7, females received a general anesthetic (xylazine (rompun), 5 mg/kg and ketamine (imalgene),

20 mg/kg, IM) and were given 2 subcutaneous implants which were empty in the control group (C, $n = 29$) and packed with crystalline progesterone (Sigma Chemical) in the treated group (group P, $n = 29$). Each implant was a 5-cm segment of silastic medical tubing (Dow Corning 601–325) sealed by medical adhesive silicone. Implants were incubated with NaCl 0.9% overnight prior to use.

Females were caged individually with a controlled light/dark cycle (16 h light/8 h dark) and free access to a standard diet (17.5% crude proteins and 2.330 kcal digestible energy per kilogram). Does and their young were weighed weekly, and feed intake was determined at that time.

Blood samples and radioimmunoassay

Blood samples were collected on d 7 (before inserting implants), and on d 16 and 25. Samples were collected in heparinized tubes by puncture in an auricular artery. They were immediately centrifuged and the plasma was stored at –20°C until assayed.

The concentration of progesterone was quantified after the extraction of progesterone from 200 µl plasma by a radioimmunoassay, as described by Thibier and Saumande (1975). The sensitivity of the assay was 0.2 ng/ml and the intra- and interassay coefficients of variation were 12 and 14%, respectively.

Reproductive performance

The does were slaughtered on d 28 of pregnancy. Their genital tract was removed and dissected. According to Adams (1960), foetuses were divided into 3 classes: i) live (L), when the foetuses were well developed and already moving or breathing; ii) resorbed (R), when the foetus was not recognizable but represented by vary-ing amount of placental tissue; and iii) dead (D), when the foetus was recognizable, but unmoving and showing marked developmental delay. The value TF is the total number of foetuses present on d 28 (TF = L + R + D). The ovulation rate was determined by counting the number of corpora lutea (CL). Foetal mortality was defined as follows:

Total mortality TM = (CL – L) x 100/CL
Early mortality EM = (CL – TF) x 100/CL
Late mortality LM = (R + D) x 100/TF

Early mortality was considered to occur during the first half of gestation and late mortality thereafter (Adams, 1960).

Live foetuses and their placentas were weighed. The does were dissected and their carcasses (muscle and bones), skin, full digestive tract, adipose tissues (perirenal and interscapular), liver, kidneys and uterine horns were weighed.

Statistical analysis

Data were analyzed by analysis of variance, using the general linear model procedures (SAS, 1987). For body characteristics of does at slaughter and ovulation rate the effect was treatment. For reproductive performance (number of foetuses and foetal mortality) the main effect was treatment and the ovulation rate (CL) was added as a covariate (model 1). The effect of the male and the treatment x ovulation-rate interactions were previously tested in this model but they were non-significant and were discarded from the final analyses. Additional analyses of foetal mortality (excepted early mortality) were made using the model 1 with TF instead of CL as a covariate (model 2). The interaction treatment x TF interaction was non-significant and was discarded from the analyses. In these models, analyses of mortality were not based on percentage but on actual numbers (*eg* CL – L for total mortality). For the weights of foetuses, placentas and uterine horns, the main effect was treatment, and the number of live foetuses was added as a covariate. For the weight of suckling litters the main effect was treatment and the litter size was added as a covariate. Live weight, feed intake and concentrations of progesterone were analyzed according to a split-plot design including the effects of treatment, stage of gestation, treatment x stage-of-gestation interaction and the effect of doe within treatment (error to test the effect of treatment). For the concentration of progesterone, the treatment x stage-of-gestation interaction was significant, therefore the effect of treatment was analyzed for each stage of gestation. Additional analyses of the concentration of progesterone were made using the effect of treatment and the ovulation rate as a covariate.

RESULTS

The mortality in the young during lactation was 4.3%, and the weight of litters was similar in both groups throughout lactation (*eg* on d 28: 4 520 ± 115 g in group P and 4 578 ± 74 g in group C).

The live weight of the does was similar in both groups at mating (3 424 ± 42 g) and throughout pregnancy. All the does gained weight from mating to d 14 (+ 605 ± 33 g), and then lost weight during the second half of pregnancy (–166 ± 21 g). There was no significant difference between does with or without progesterone implants either in feed intake during pregnancy (364 ± 4 g/d) or in body composition at slaughter (table I).

On d 7 the concentration of progesterone was similar in both groups (fig 1). It was 29.4% higher in group P on d 16 (15.4 ± 0.8 ng/ml *vs* 11.9 ± 0.6 ng/ml; $P < 0.01$) and 41.4% higher on d 25 (14.0 ± 0.7 ng/ml *vs* 9.9 ± 0.7 ng/ml; $P < 0.01$). The variability in the concentration of progesterone was similar in both groups, and the repartition curves of the progesterone concentration in the 2 groups, were partly superposed (fig 2). At each stage of gestation, the concentration of progesterone was positively related to the ovulation rate (coefficient of regression: CR = 0.55, 0.94, 0.72 on d 7, 16 and 25, respectively; $P < 0.05$).

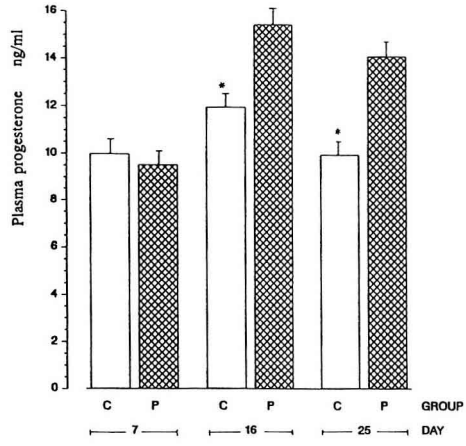

Fig 1. Concentration of progesterone (ng/ml) in progesterone-supplemented (P) and control (C) does at different stages of pregnancy. The asterisks indicate a difference between experimental and control group within a stage of pregnancy ($P < 0.01$).

Table I. Body composition of does slaughtered on d 28 of gestation.

	Group [a]		
	C	P	SEM [b]
No of pregnant does	29	29	–
Live weight (g)	3 703	3 769	41
Carcass weight (g)	1 887	1 925	23
Skin weight (g)	489	486	8.8
Digestive tract weight (g)	515	509	10.5
Adipose tissues weight (g)	26	25	2.1
Uterine weight (g)	44	44	1.3
Liver weight (g)	116	116	2.4
Kidney weight (g)	19.3	19.9	0.3

[a] C: control progesterone group; P: progesterone-implanted group; [b] standard error of the mean.

The weight of foetuses, placentas and uterine horns were similar in both groups. The ovulation rate were also similar (11.2 ± 0.3 in group C and 11.1 ± 0.3 in group P). Late and total mortality, as well as the number of dead or resorbed foetuses were not significantly different in both groups (table II). There were 0.9 more live foetuses in group P than in C, but the difference was not significant. Early mortality was lower ($P < 0.05$) and TF was higher ($P < 0.05$) in group P than in C. The number of live foetuses and TF were positively related to the ovulation rate whatever the group (CR = 0.77 and 0.75, respectively; $P < 0.001$). In contrast, other variables (number of dead or resorbed foetuses and foetal mortality) were not significantly related to the ovulation rate. Owing to the higher TF in group P, late and total mortality were adjusted for this variable

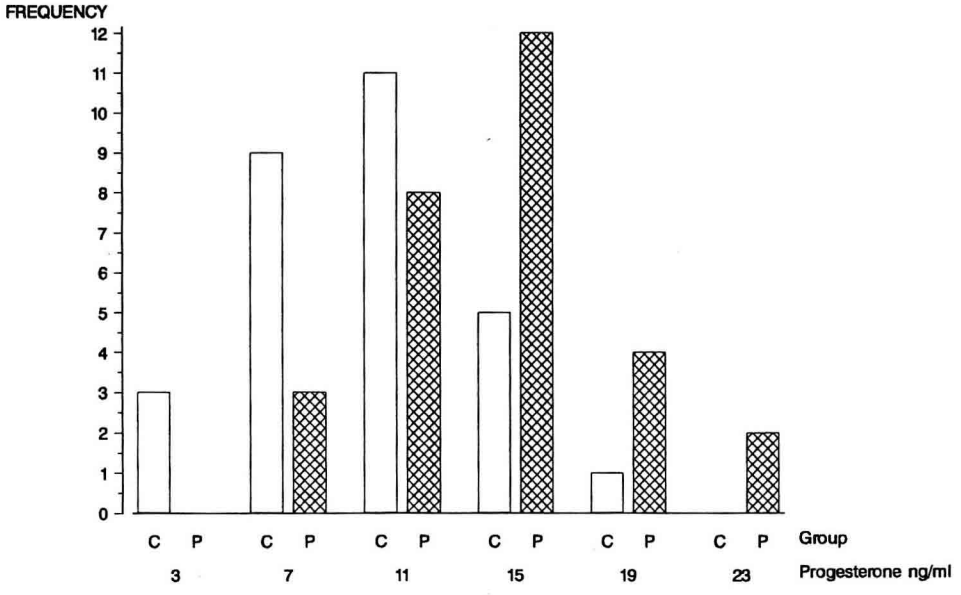

Fig 2. Distribution curve of the concentration of progesterone in groups P and C on d 25.

Table II. Reproductive performance of does slaughtered on d 28 of gestation.

	Group [a]		
	C	*P*	*SEM* [b]
No of pregnant does	29	29	–
No of corpora lutea	11.2	11.2	0.3
No of foetuses			
Total [c]	8.7	9.8 *	0.4
Live	8.0	8.9	0.4
Resorbed	0.5	0.7	0.2
Dead	0.2	0.2	0.1
Total mortality [d] (%)	27.5	21.0	3.6
Early mortality [d] (%)	21.2	12.3 *	3.2
Late mortality [d] (%)	10.8	9.5	3.2
Foetal weight (g)	36.1	35.3	0.7
Placental weight (g)	7.5	7.2	0.2

Values are least-square means; [a] C: control progesterone group; P: progesterone-implanted group; [b] standard error of the mean; [c] live + resorbed + dead foetuses; [d] for calculation see text; * groups differ at $P < 0.05$.

in further analysis (model 2). Nevertheless, when TF was taken into account instead of the ovulation rate, the progesterone treatment had no significant effect either on late or total mortality.

DISCUSSION

The use of progesterone implants allowed a progressive liberation of the hormone and its concentration in treated lactating does was restored to levels observed in non-lactating pregnant does (Fortun *et al,* 1993). The level of progesterone supplementation was physiological and a variability in the concentration of progesterone also remained in the progesterone-supplemented group. Besides, similar growth in the suckled young suggested similar milk production in both groups of does.

A direct anabolic effect of progesterone has been demonstrated in pregnant rats by

Shirling *et al* (1983). In our experiment, the slight increase in the concentration of progesterone neither modifies the live weight nor the body composition of does at slaughter. Furthermore, foetal growth was similar in lactating does whether supplemented with progesterone or not.

Progesterone supplementation led to an increased total number of foetuses at the end of pregnancy with no effect on late mortality. Thus, progesterone level seems to affect early rather than late pregnancy, if the hypothesis of a similar number of embryos in both groups before implants were inserted is true. These results closely agree with those obtained with ewes (Davis *et al,* 1986; Kleeman *et al,* 1991), gilts (Ashworth, 1991) and hamsters (Pratt and Lisk, 1991). It should be noted that early mortality in the control groups was relatively high compared with previous experiment results (Fortun, 1993; Fortun *et al,* 1993), whereas it is in the same range as previous results from the same genetic line (Bolet *et al,* 1990).

Effects of progesterone levels on late foetal survival is less documented than effects on embryo survival. In pregnant hamsters exposed to social subordination, implantation failure and foetal resorption are correlated with a reduced concentration of progesterone (Huck *et al,* 1988). Our experiment did not show any significant influence of progesterone supplementation on the number of resorbed and dead foetuses. However, late foetal mortality could be due to events occurring in early pregnancy. In this case, the insertion of the progesterone implants on d 7 could be too late.

In a previous experiment, higher foetal mortality and lower progesterone concentrations were observed in pregnant does treated with prolactin (Fortun, 1993). Furthermore, Lin *et al* (1987) have shown that hyperprolactinemia inhibits steroidogenesis in rabbit does. Thus, high levels of prolactin during lactation seem to be responsible for lower progesterone concentrations in simultaneously pregnant and lactating does (Fortun *et al,* 1993). Nevertheless, present results indicate that the decrease in progesterone concentration is associated with the decrease in foetal survival in lactating does, but is not responsible for this. Thus, the hyperprolactinemic status of lactation may have a direct effect on foetal survival. Specific receptors for prolactin have been described in rabbit uterine membranes (Tamaya *et al,* 1980; Ohno, 1982). Moreover, injections of prolactin can induce modifications in the quality of uterine secretions of protein (Chilton and Daniel, 1987; Daniel *et al,* 1988). Thus, high prolactin levels during lactation could change relations between the uterus and the foeto-placental unit, inducing foetal mortality.

These results indicate that progesterone supplementation cannot prevent the increased late mortality that occurs in concurrently pregnant and lactating does. Further experiments are needed to clarify the influence of prolactin on foetal mortality in lactating does.

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REFERENCES

Adams CE (1960) Studies on prenatal mortality in the rabbit, *Oryctolagus cuniculus*: the amount and distribution of loss before and after implantation. *J Endocrin* 19, 325-344

Ashworth CJ (1991) Effect of premating nutritional status and post-mating progesterone supplementation on embryo survival and *conceptus* growth in gilt. *Anim Reprod Sci* 26, 311-321

Bolet G, Brun JM, Hulot F, Theau-Clément M (1990) Variabilité génétique et effet de la sélec-

tion dans le croisement de trois souches de lapin –2– composantes biologiques de la taille de portée. *5es Journ Rech Cunicoles,* Comm 65

Challis JRG, Davies IJ, Ryan KJ (1973) The concentrations of progesterone, estrone and estradiol-17β in the plasma of pregnant rabbits. *Endocrinology* 93, 971-976

Chilton BS, Daniel JC (1987) Differences in the rabbit uterine response to progesterone as influenced by growth hormone or prolactin. *J Reprod Fertil* 79, 581-587

Daniel JC, Juneja SC, Taylor SP, Lonergan PB, Sullivan PK, Chilton BS (1988) Variability in the response of the rabbit uterus to progesterone as influenced by prolactin. *J Reprod Fertil* 84, 13-21

Davis KR, Kerton DJ, Parr RA, White MB, Williams AH (1986) Hormone supplementation to increase fertility after uterine insemination in ewes. *Proc Aust Soc Anim Prod* 16, 171-175

Fortun L (1993) Effects of prolactin on fetal survival in concurrently pregnant and lactating rabbits does. VI. International Prolactin Congress, Paris, 30 August–3 September

Fortun L, Prunier A, Lebas F (1993) Effects of lactation on fetal survival and development in rabbit does mated shortly after parturition. *J Anim Sci* 71, 1882-1886

Herrenkohl LR (1972) Effects on lactation of progesterone injections administered after parturition in the rat. *Proc Soc Exp Biol Med* 40, 1356-1359

Holt JA (1989) Regulation of progesterone production in the rabbit corpus luteum. *Biol Reprod* 40, 201-208

Huck UW, Lisk RD, Miller KS, Bethel A (1988) Progesterone levels and socially induced implantation failure in golden hamsters. *Behav Neural Biol* 38, 190-193

Kleemann DO, Walker SK, Grimson GJ, Smith DH, Grosser TI, Seamark RF (1991) Exogenous progesterone and embryos survival in Booroola-cross ewes. *Reprod Fertil Dev* 3, 71-77

Lin KC, Okamura H, Mori T (1987) Inhibition of HCG-induced ovulation and steroidogenesis by short-term hyperprolactinemia in female rabbits. *Endocrinol Jpn* 34, 675-683

Ohno Y (1982) Studies on the interaction of prolactin and estrogen in rabbit ovary and uterus. *Acta Obstet Gynaecol Jpn* 34, 252-260

Pratt NC, Lisk RD (1991) Role of progesterone in mediating stress-related litter deficits in the golden hamster *(Mesocricetus auratus). J Reprod Fert* 92, 139-146

SAS (1987) SAS User's Guide: Statistics SAS Inst Inc, Cary, NC

Schacht CJ, Foote RH (1978) Progesterone-induced asynchrony and embryo mortality in rabbits. *Biol Reprod* 19, 534-539

Shirling D, Ashby JP, Baird JD (1981) Effect of progesterone on lipid metabolism in the intact rat. *J Endocrin* 90, 285-294

Shirling D, Ashby JP, Baird JD (1983) A direct anabolic effect of progesterone in the intact female rat. *J Endocrin* 99, 47-50

Tamaya T, Ohno Y, Ide N, Tsurusaki T, Okada H (1980) Occurrence of prolactin binding sites in rabbit ovary and uterus. *Jpn J Fert Steril* 25, 8-12

Thau RB, Lanman T (1974) Evaluation of progesterone synthesis in rabbit placentas. *Endocrinology* 94, 925-926

Thau RB, Lanman T (1975) Metabolic clearance rates (MCR) and production rates (PR) of plasma progesterone in pregnant and pseudopregnant rabbits. *Endocrinology* 97, 454-457

Thibier M, Saumande J (1975) Oestradiol-17β, progesterone and 17α-hydroxyprogesterone concentrations in jugular venous plasma in cows prior to and during oestrus. *J Steroid Biochem* 6, 1433-1436

Torrès S (1982) Étude de la mortalité embryonnaire chez la lapine. *3es Journ Rech Cunicoles,* Comm 15

Zoldag L, Gabor GY, Molnar L, Solti L (1988) Progesterone concentrations of New-Zealand rabbits in varied reproductive phases. *4th World Rabbit Congress,* 486-492