EFFECTS OF CONCURRENT GESTATION AND LACTATION IN RABBIT DOES ON POSTNATAL DEVELOPMENT AND MUSCULAR CHARACTERISTICS OF THE YOUNGS. PRELIMINARY RESULTS

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Abstract - The aim of this experiment was to determine the influence of concurrent gestation and lactation in rabbit does on the postnatal growth and muscular characteristics of the progeny. Young rabbits from either simultaneously pregnant and lactating does (n=14, E group) or only pregnant does (n=15, C group) were weighted from birth to slaughter age. Myosin heavy chain (MHC) isoform proportion was determined on day 29 or day 70 (commercial slaughter age) in the *semitendinosus* muscle of the youngs. The weight was similar in the two groups form birth to slaughter age. On day-29, the proportion of perinatal MHC was higher (5.8% vs 2.3%) and the type-II isoform content was lower (91.5% vs 95.0%) in the E group than in the C group. On day-70, no differences were seen between the two groups on the proportion of the various isoforms Our results suggested that concurrent gestation and lactation did not depress postnatal growth of the progeny but delayed the myofibre maturation rate. The mechanisms of the effect of lactation on muscle maturation remained to be clearly elucidated.

INTRODUCTION

The female rabbit can be fertilized shortly after parturition and be simultaneously pregnant and lactating. But voluntary feed intake of primiparous does is insufficient to supply all the nutritional requirement: maternal tissue growth, foetal development and milk production (Fortun-Lamothe and Lebas, 1995). This induces a competition between uterus and mammary gland for nutrient supply and gestation and lactation are therefore concurrent. Preliminary studies have shown that foetal growth (observed on day 28 of gestation) are lower in lactating than non lactating does (-19.6%, Fortun *et al.*, 1993). Repercussion of the lower foetal growth on postnatal growth and muscular characteristics of the youngs is not well known.

Skeletal muscles consist mainly of muscular fibres differing in their biochemical and physiological characteristics. The type of myofibres is one of the factors that affect the transformation of muscle into meat (Valin, 1988). At a molecular level, the myofibre types are related to the content in Myosin Heavy Chains (MHCs) composition. In the rabbit skeletal muscles, two developmental (embryonic and perinatal) and four adult (I, IIa, IIb and IIx) MHC isoforms have been identified (JANMOT and D'ALBIS, 1994). During foetal and early postnatal periods, immature muscles are transformed into efficiently mature units. Particularly, expression of developmental isoforms is gradually suppressed while the expression of adult isoforms is enhanced (Schiaffino *et al.* , 1989). The proportion of the different isoforms (developmental and adult) in the muscle can be then used as a marker of muscle maturity.

Acquisition of mature phenotype is controlled by complex regulation mechanisms, including nervous and hormonal factors (for review, see Vigneron *et al.* , 1989). In addition, muscle fibre growth could be influenced by environmental factors, such as nutrient intake level during foetal or postnatal period (Ward and Stickland, 1993, Dwyer and Stickland, 1994).

The aim of this work is to study the effects of concurrent gestation and lactation in rabbit does on the postnatal growth and muscular characteristics of the youngs at weaning (29 days) and commercial slaughter age (70 days).

MATERIALS AND METHODS

Animals

Eigth 24-weeks-old Californian x New Zealand does were assigned at their first parturition to one of the two experimental groups according to their litter size and body weight. In the experimental group (E group) the females (n=4) were presented to the male within 24 hours after parturition (simultaneously pregnant and lactating does). In this group, the second pregnancy is therefore concurrent with lactation. The young rabbits of

this second pregnancy were used to study muscular development (E group). In the other group, the females (n=4) were not remated after parturition (pregnant non lactating does) and the young rabbits of the first parturition were used as control (C group). In the two groups, the suckling rabbits were weaned on day 28 of lactation. The animals (females and weaned rabbits) had free access to water and to a commercial diet (17.5% crude protein and 2330 kcal digestible energy/kg).

Growth performance

Twenty-nine young rabbits (n=14 and 15 in groups E and C, respectively) were weighted every week from parturition to slaugther, on day 29 (weaning) or 70 (commercial slaughter stage).

Muscle sampling and electrophoresis of myosin heavy chains (MHC) isoforms

Five animals from each group were killed at 29 or 70 days of age (by electric stunning and exsanguination). The muscle *Semitendinosus* (white portion) was removed and was frozen in isopentane cooled with liquid nitrogen. Electrophoresis was performed according to the method described by Janmot and d'Albis (1994). Quantification of the different isoform proportion was obtained by densitometry.

Statistical analysis

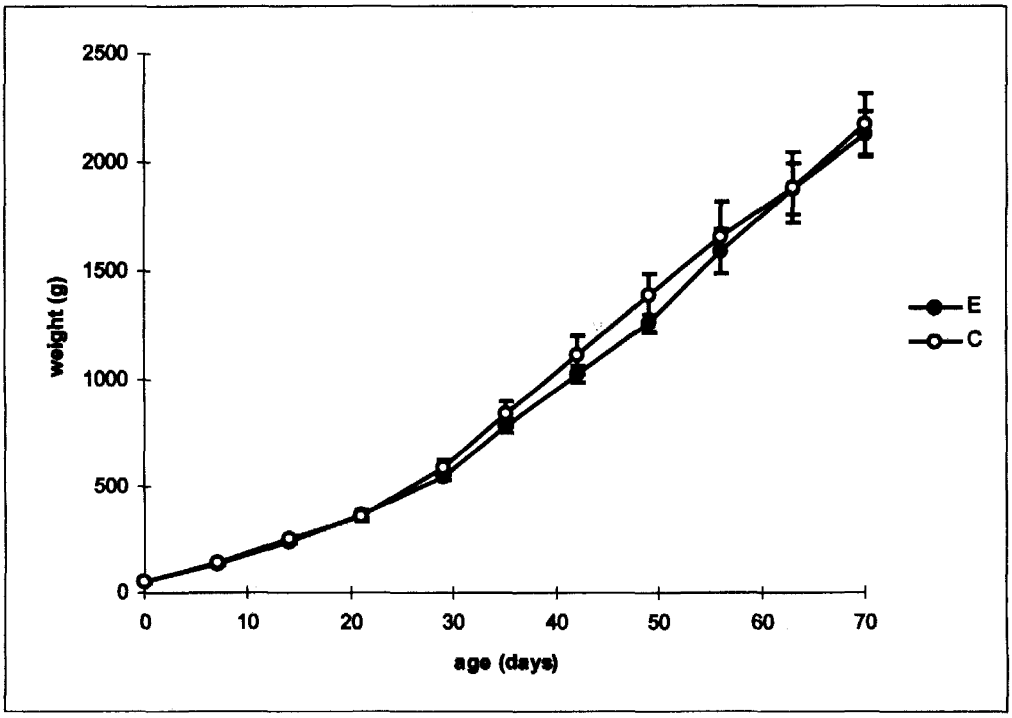
Mean and standard deviations of the means were calculated from individual values by the usual procedures (SAS-Package, 1990). Analysis of variance were performed with treatment as main effect. Differences were considered significant at $P < 0.05$.

RESULTS

Growth performance

Body weight curves of the young rabbits of the two groups from birth to 70 days of age are shown in fig. 1. Birth weight was 9% lower in the E group than in the C group. However, postnatal growth performance was not significantly different between E and C groups (P>0.05).

Figure 1 : Body weight curves of youngs from simultaneously pregnant and lactating does (E group) or from pregnant does (C group) during postnatal period.



Proportion of the different MHC isoforms

At day 29, five isoforms could be identified on the gels, i.e. MHC-perinatal, MHC-IIa, MHC-IIx, MHC-IIb and MHC-I. The major difference between the two groups was in the perinatal MHC isoform content. The proportion of MHC-perinatal was significantly greater in E group (5.8% of the total isoform content vs 2.3%, P=0.0006) (table 1). Type II (i.e. IIa+IIb+IIx) isoform content was lower in this group (91.5% vs 95%, P=0.005). On the opposite, the amount of type I isoform was not affected by treatment (P=0.9). At day 70, the perinatal isoform was no longer expressed and the proportion of the different adult isoforms was similar in the two groups (table 1).

Table 1 : Proportion of the various MHC isoforms in the *semitendinosus* muscle of youngs from simultaneously pregnant and lactating does (E group) or from pregnant does (C group).

	29 days		70 days	
isoforms	E-group	C-group	E-group	C-group
perinatal	5.8 ± 0.6 ***	2.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
IIa	8.1 ± 2.1	8.5 ± 0.7	11.5 ± 1.5	7.3 ± 2.0
IIx	49.4 ± 2.2	50.3 ± 1.9	49.5 ± 7.5	52.2 ± 2.6
IIb	34.0 ± 4.7	36.1 ± 2.6	33.0 ± 8.0	37.0 + 2.3
I	2.7 ± 1.1	2.6 ± 0.6	6.0 ± 2.0	3.5 ± 1.6
total of type II	91.5 ± 1.6 **	95.0 ± 0.9	94.0 ± 2.0	96.5 ± 0.6

** P ≤ 0.01 *** P ≤ 0.001

DISCUSSION

These results suggest that concurrent pregnancy and lactation do not affect postnatal growth of the litter but delay the maturation of the muscular fibres of the youngs.

Fortun *et al.* (1993) have previously shown that foetal growth is reduced in lactating compared to non lactating does (-19.6% on day 28 of gestation). In the present study, the difference in the weight of the youngs of the two groups observed at birth was lower (-9%) and was not significant. That could be due to the small groups size (n=14 and 15 in groups E and C, respectively). Foetuses of simultaneously pregnant and lactating does could also have more important growth (catch-up growth) during the last days of gestation (days 28 to 32), when does are no more suckling. Thereafter, postnatal growth of the youngs seems to be not affected by simultaneous pregnancy and lactation.

The perinatal MHC isoform content can be used as an indicator of muscular immaturity. At day 29 of age, the perinatal MHC proportion was higher in the *semitendinosus* muscle of youngs from the E group, denoting a less mature state. This result suggests that the maturation of muscular fibres is impaired in the progeny from simultaneously pregnant and lactating does. In this situation, the expression of type II isoforms seems to be delayed, while type I isoform expression seems to be not affected. Effects of concurrent pregnancy and lactation observed on day-29-old youngs must have occured before birth because, during suckling, the experimental conditions were similar in both groups. Therefore, two hypotheses can explain this impairment of myosin composition observed: when does are simultaneously pregnant and lactating, the nutrient requirements of the foetuses are not satisfied and (or) concurrent lactation induces an hormonal environment that is unfavourable for muscular development of the youngs.

Previous studies have shown that energetic balance during the 0-28 days of gestation is highly negative in does which are simultaneously pregnant and lactating (-11.78 MJ), whereas it is positive in pregnant and not lactating does (+12.51 MJ ; Fortun and Lebas, 1994). To limit their energy deficit, lactating does increase their feed intake (354 ± 7 g/j vs 199 ± 8 g/j in control does) (Fortun and Lebas, 1994) but this increase is not sufficient enough. Therefore, the nutritional deficit in simultaneously pregnant and lactating does induces a competition between uterus and mammary gland for nutrient supply, which is detrimental to foetal growth (FORTUN *et al.*, 1994). It has been previously shown in rodents that maternal undernutrition during pregnancy can affect the muscular development of the youngs (Dwyer and Stickland, 1994). Therefore, the nutritional deficit induced by lactation could explain the delayed muscular development observed at weaning in the progeny of simultaneously pregnant and lactating does. In guinea-pigs, severe maternal undernutrition during pregnancy affect preferentially secondary fibres (type II fibres in fast muscles), than type I fibres (Dwyer and Stickland, 1994). In rabbit species, our observations are in agreement with these results. It is suggested that type

II myosins could be more labile to nutritional influences *in utero*, than type I myosin, as previously noticed about muscular fibres in pigs (Stickland, 1995).
In other hand, uterine environment is greatly different in simultaneously pregnant and lactating does and in pregnant non-lactating does. For exemple, insulinemia was found to be lower in pregnant and lactating does than in pregnant does (FORTUN, 1994). Maternal insulinemia during gestation influences foetal growth modifying the placental transfert of glucose (BATTAGLIA and MESHIA, 1988; HAY *et al.*, 1985) and could therefore modify the muscular development of the foetuses. The level of several other hormones is changed during lactation (COLLIER *et al.*, 1984). However, most of the protein hormones (even a peptide as small as thyroxine) do not traverse the placenta from maternal to foetal blood. Therefore, the influence of the hormone level changes during lactation on muscular development of the foetuses remains to be elucidated.
In our experiment, no differences were seen between the two groups about the adult isoform proportion at day 70 in *semitendinosus* muscle, suggesting that concurrent lactation and gestation did not alter definitively the myofibre type proportion of the progeny. This is in aggrement with NordBy *et al.* (1987), who have shown that maternal dietary restriction during pregnancy did not affect fibre type proportion in *semitendinosus* muscle of postweaning lambs. On the opposite, our results are not consistent with some prenataly dietary restriction experiments (-50% of the maternal volontary feed intake), which have evidenced that fibre type proportion may be altered in the adult rats (Bedi, 1982). But it is difficult to compare directly the results presented in these experiments with ours. This is due to differences in the intensity of undernutrition, timing of the imposed period of undernutrition, species and muscles.
In other hand, BEDI (1982) has shown that prenatal undernutrition caused permanent deficit in fibre cross-sectional areas. Therefore, effects of lactation on fibre measurements remain to be clarified.

CONCLUSION

Our study has shown that concurrence between gestation and lactation altered some of the muscular characterictics of the progeny. It induced a delay in the rate of maturation of the myofibres. However, no remnant effects were seen at commercial slaugther age, either on growth performance or fibre type proportion. The eventual effects of lactation on the other muscular characteristics (fibre size, total number of myofibres, protein content of the muscle) remain to be investigated. The mechanisms implicated in the effects of lactation on muscular development remain to be clearly elucidated.

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Effet de concurrence entre la gestation et la lactation chez la mère lapine sur le développement post-natal et les caractéristiques musculaires de leurs lapereaux. Premiers résultats - Cette expérience a été mise en place afin de déterminer l'influence d'une gestation et d'une lactation simultanées chez la lapine sur la croissance postnatale et les caractéristiques musculaires de la portée. 29 lapereaux, nés de femelles gestantes-allaitantes (n=14, lot E) ou de femelles uniquement gestantes (n=15, lot C), ont été pesés de la naissance à l'abattage. La proportion des différentes isoformes des chaînes lourdes de la myosine a été déterminée à 29 jours ou à 70 jours (stade commercial d'abattage) dans le muscle *semitendinosus* des lapereaux. A 29 jours, la proportion de l'isoforme périnatale est plus élevée (5,8% vs 2,3%, P<0.001) tandis que la proportion des isoformes de type II est plus faible (91.5% vs 95.0%) dans le groupe E, comparé au groupe C. A 70 jours, il n'y a pas de différence entre les deux groupes dans la proportion des isoformes de myosine. Les résultats suggèrent que la concurrence entre la gestation et la lactation n'affecte pas les performances de croissance des jeunes mais induit un retard dans la vitesse de maturation des fibres musculaires. Les mécanismes impliqués dans les effets de la simultanéité de la gestation et de la lactation sur la maturation du muscle restent à déterminer clairement.