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IMPACT ON MUSCLE FIBER PROPERTIES OF MATERNAL NUTRIENT RESTRICTION FOLLOWED BY REALIMENTATION FROM EARLY TO MID-GESTATION IN BEEF COWS

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Abstract – Muscle fiber properties are important for beef meat production: quantity and quality. Fetal life represents crucial phase for the acquisition of muscle properties. Conditions of gestation of the cow will have impacts on muscle development. Very few studies concern the effect of nutrition restriction during gestation in cattle. Our objective was to analyze the impact of nutrient restriction at specific gestational stages on fiber properties of *Semitendinosus* muscle. Cross bred beef cows were fed to nitrogen and energy recommendations (CCC) or restricted to 60% of recommendations for the first 85 or 140 days post conception followed by realimentation to recommended protein and energy levels (RCC and RRC respectively). The morphology and properties of fibers were analyzed by immunohistochemistry with specific anti myosin heavy chains antibodies. The main effects of undernutrition were observed at 254 days. The most important effect is the presence of lower proportions of small fibers expressing fetal myosin heavy chain in RCC and RRC groups in comparison to CCC. As these fibers correspond to the third generation giving rise to IIA fibers, this could have consequence on the proportion of IIA fibers of this muscle.

Key Words – skeletal muscle, myogenesis, growth fetal programming

I. INTRODUCTION

In farm animals, muscle fiber properties play a key role in meat quality. So understanding the growth and development of skeletal muscle is one of the most important goals in meat production science. Myogenesis has been well documented in cattle (for review: Picard *et al.* [1], [2]). This is a continuous process involving hyperplasia and hypertrophy of cells. Different studies of bovine fetuses showed that the total number of fibers is fixed at about the end of the second trimester of gestation [1]. Fiber size increases from this point as observed also in sheep [3]. This process continues during perinatal and postnatal life [2]. The properties of bovine muscle fibers are acquired in a three generation process (for a review: [1]). Primary generation observed from 30 days of fetal life is precursor of slow fibers which are completely differentiated by the end of the second trimester (around 180 d). A secondary generation is observed from the end of the first trimester, and mostly matures to fast IIX fibers. A third generation observed from 40% of the gestation period onwards as in sheep and human [3] gives rise to fast IIA fibers, slow I fibers and IIC fibers. The analysis of different differentiation markers showed that contractile and metabolic differentiation occurred during the last trimester [4]. The effects of the manipulation of maternal nutrition on muscle fiber properties have been studied in sheep but rarely in cattle. In ewes effects of maternal undernutrition (50 % of intake of controls) on muscle development were addressed at three different periods of pregnancy around the primary fiber formation, the secondary fiber formation or the post-fiber formation. The most critical period was before the peak in secondary fiber formation in ewes (days 30-70). It resulted in increased diameter of fast fibers, no change in diameter of slow fibers and decrease of fast-to-slow fibers ratio per unit area [5]. Elsewhere, in ewes undernutrition between days 85 to 115 of gestation after the period of fiber formation had no effect on the number of fibers in the newborn lambs, but decreased the weight of muscles [6]. Whatever the species, it clearly appears that the effects of maternal nutrition depend on the stage of gestation [3].

The objective of the present study was to evaluate the effects of nutrient restriction during early gestation followed or not by realimentation to

mid-gestation on muscle fibers properties in bovine.

II. MATERIALS AND METHODS

Semitendinosus (ST) muscle was sampled from bovine fetuses at 140 and 254 days post-conception (dpc) as previously described [7]. Briefly, Angus cows were randomly assigned to three dietary treatments at 30 days of pregnancy: cows maintained at 100% nitrogen and energy (NE) recommendations for maintenance and fetal growth from 30 to 140 dpc (CC, n= 5), cows nutrient restricted to 60% NE recommendations until 85 dpc followed by realimentation to 100% NE recommendations up to 140 dpc (RC, n=5) and cows nutrient restricted to 60% NE from 30 to 140 dpc (RR, n= 5). On d 140, cows were slaughtered (CC, n = 5; RR, n = 5; RC, n = 5), remained on control (CCC, n = 5; RCC, n = 5), or were realimented to control (RRC, n = 5). On d 254, all remaining cows were slaughtered.
Fiber types, number and cross-sectional areas were analyzed by immunohistochemistry with specific antibodies as described in Duris *et al.* [8]. Muscle samples for immunohistochemistry were frozen progressively in isopentane cooled by liquid nitrogen and stored at –80°C until analysis.

Immunohistochemistry

Transverse serial sections of ST muscle, 10 µm in thickness, were obtained using a cryostat microtome at -25°C. The myosin heavy chain isoforms (MyHC) were revealed with different antibodies: F365 B9 specific to slow MyHC; F113 15F4, specific to all the fast MyHC in adult muscle and labels a developmental isoform during fetal life; F158 4C10 raised against a fetal MyHC; and S5 8H2 which recognises all adult MyHCs with the exception of MyHC IIa. All of these antibodies were obtained from Biocytex (Marseille, France). Their specificity and conditions for use in bovine muscle have been validated and described previously [9]. Sections were observed and micro-photographed under a microscope equipped with a camera. Cell density and morphology were observed. The response of the antibodies was examined on a minimum of 200 fibers on each serial section.

Anova test was realized with R software to analyze the differences between the three nutritional groups.

III. RESULTS AND DISCUSSION

Few differences were observed between groups at 140 dpc. More differences were visible at 254 dpc corresponding to a differentiation and maturation of muscle fibers stage.

140 dpc

At 140 dpc, fibers from the primary generation were labelled with 5B9 antibody specific to adult slow MyHC (Figure 1). At this stage secondary fibers do not contain slow MyHC [1, 2]. The ratio (r) of secondary/primary fibers was not significantly different between the 3 groups (CC r=8, RC r=8.4, RR r=8.7). Several authors observed a reduction in secondary fiber number reflected in a reduced secondary/primary fiber ratio induced by early gestational nutrient deprivation. The difference with the present study could be explained by muscle specific effect.

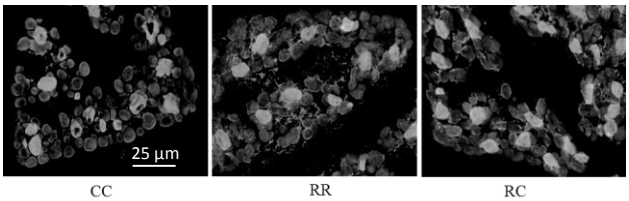

Figure 1. Labelling with 5B9 antibody (slow MyHC I).
Fibers from the first generation are in white, the second generation is in grey. Magnification is the same for the three groups.

Despite a cross sectional area of fibers slightly superior for RCC group (Figure 2), the difference between the 3 groups was not significant. However, these preliminary results need to be completed with more numerous samples. In *Infraspinatus* muscle Gonzalez *et al.* [7] showed that RCC group had higher cross sectional area of fibers by comparison to CCC and RRC. They explained these differences by compensatory growth induced by realimentation of pregnant dam

after 85 dpc. These differences in fiber size were no longer observed at 254 dpc, as in the present study for ST muscle (data not shown).

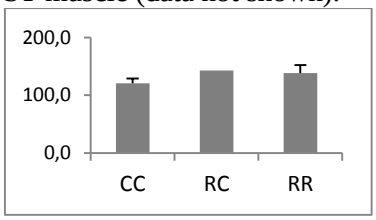

Figure 2. Mean cross sectional area of fibers (μm^2)
n= 5 CC, 1 RC, 3 RR

The labelling of the four antibodies used on serial sections allowed the distinction of 4 types of fibers at this stage. According to Picard *et al.* [1] all contained fetal MyHC. Among the second generation, 3 types of fibers were distinguished with differences in the proportions of fetal, embryonic and alpha cardiac MyHCs [1]. In the present study no significant differences were observed between the 3 groups in the proportions of these different fibers (data not shown).

254 dpc

At 254 dpc numerous different types of fibers were observed in accordance with Picard *et al.* [1]. Slow fibers from the first generation no longer expressed development MyHC isoforms. Among the secondary population of fibers, we could distinguish pure fibers I, IIA and IIX and hybrid fibers (IIC and IIAX). A proportion of these fibers still expressed developmental MyHCs and others had stopped to express these isoforms (Table 1).

Table1 Proportions of the different types of fibers observed at 254 dpc in the 3 groups.
F: fetal MyHC. Fibers containing I and IIA MyHC correspond to hybrid IIC fibers.

Fibres %	CCC	RCC	RRC
I	8.0	7.5	6.7
I-F	10.4	9.3	16.8
IIX	27.8	26.2	26.9
IIX-F	8.1	11.8	13.7
IIA	7.4 [a]	1.6 [b]	8.3 [c]
IIA-F	27.6	33.4	21.3
I-IIA	0.0 [a]	0.7 [b]	0.0 [a]
I-IIA-F	0.0	4.1	0.4
IIA-IIX	4.5	1.0	1.1
IIA-IIX-F	0.5	0.7	0.1
F	5.6	3.7	4.7

a, b, c illustrate the significant statistical differences between the 3 groups, P<0.05.

The proportion of I-F fibers was higher in RRC but not significantly certainly because of the large individual variability (Table 1). This could illustrate a delay in the differentiation of slow fibers in RRC group. The most important differences between the 3 groups concerned IIA fibers (P=0.033). The proportion of IIA fibers was lower in RCC group. The proportion of IIA fibers still containing fetal MyHC was higher in this RCC group (Table 1). This could illustrate a delay in the differentiation of IIA fibers.

A population of very small fibers labelled with anti fetal MyHC antibody not accounted in Table 1, were observed (Figure 3).

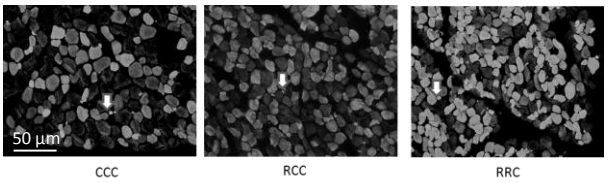

Figure 3. Labelling with 4C10 antibody specific of fetal MyHC.
White arrows indicates the very small fibers labelled with the anti fetal MyHC antibody. Magnification is the same for the three groups.

These fibers have been previously described by Picard *et al.* [1] as a third generation of fibers giving rise to fast IIA fibers and slow I and IIC hybrid fibers. In the present study the proportion of these fibers was two fold higher in CCC (9.6%, P=0.014) in comparison to RCC (4.7%, P=0.00715) and RRC (5.1%, P=0.0226) groups. No differences were observed between RCC and RRC (P=0.974). These results are in accordance with the differences observed in the proportions of IIA and IIA-F fibers (Table 1). This suggests that undernutrition could affect the proliferation and differentiation of the third generation precursor of IIA fibers. Globally, the proportions of fibers still containing fetal MyHC was higher in RCC (64%) comparatively to CCC (52%) and RRC (57%), in coherence with a delay in the differentiation of fibers after maternal undernutrition.

IV. CONCLUSION

This *in vivo* investigation about the effect of maternal nutrient restriction on bovine myogenesis revealed some differences in fiber properties. The differences seem to be more important at the end of fetal life. This suggests that modifications of maternal nutrition could have more impacts on fiber differentiation than on their proliferation. Further analyses are in progress to complete and validate these preliminary results.

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