

EFFECTS OF pST ADMINISTRATION TO PREGNANT SOWS ON DEVELOPMENTAL STAGE AND SEMITENDINOSUS MUSCLE CELLULARITY OF THE NEWBORN PIGLETS

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SUMMARY: Pregnant Landrace sows were treated with 6 mg porcine somatotropin (pST) per day during different periods of gestation to investigate the effects on the developmental stage of their piglets at birth. The treatment period was between 10 and 24 (I), 50 and 64 (II) and 80 and 94 (III) days of gestation, respectively. In response to the treatment in the late pregnancy (III) the birthweight of the piglets increased by 4.8% ($P < 0.05$). The mean litter size was unchanged. Organs, with the exception of spleen, pancreas, and thyroid gland tended to be heavier in group III (by 9 to 33%), and the body contained more fat ($P < 0.05$). There was an increase in *semitendinosus* muscle weight, although changes on the cellular level were not evident. Group III showed less nuclei per muscle fibre ($P < 0.05$) and the nuclei number per mm^2 after DNA concentration tended to be lower, whereas RNA concentration and RNA/DNA ratio were higher compared to controls. Taken together with the findings that this group showed the largest fibre number per Type I cluster ($P < 0.05$) and the most compact fibre arrangement, the results indicate a higher maturity of skeletal muscle in Group III. In Group I the total muscle fibre number was enhanced by 27%, i.e. by 80,000 fibres. The results suggest that pST treatment of sows during gestation may accelerate the development of the fetuses and may induce the formation of more muscle fibres representing a higher growth capacity of skeletal muscle. The effects of elevated maternal somatotropin on the fetus are assumed to be mediated by improved nutrient availability as well as fetal insulin and insulin-like growth factors.

INTRODUCTION: Administration of somatotropin to pigs is associated with an increased growth rate, altered body composition and increased efficiency of feed utilization (MACHLIN, 1972; ETHEBERTON et al., 1987). In part this is due to the metabolic effects of somatotropin which are catabolic and lead to enhanced availability of carbohydrates, fatty acids and amino acids (ETHEBERTON, 1988). In particular, somatotropin is capable of increasing plasma glucose and insulin (GLUCKMAN, 1986). Since substrate availability is one of the most important factors for fetal growth, it can be expected to be influenced by exogenous administration of somatotropin to the maternal organism. Moreover, an indirect mitogenic effect of maternal somatotropin is imaginable, if nutrient and in particular glucose availability is a significant determinant of fetal IGF secretion and if IGF plays a role in the regulation of fetal growth, as documented by GLUCKMAN et al. (1986) and BASSETT et al. (1990). This is of particular interest, because the number of myoblasts may be the limiting factor for muscle fibre number, which is generally fixed at birth (e.g. REHFELDT et al., 1987). Thus prenatal factors which affect total muscle fibre number have a permanent effect on the postnatal growth (WIGMORE and STICKLAND, 1983). Consequently, the aim of this study was to investigate the effects of skeletal muscle somatotropin (pST) administered to pregnant sows on the developmental stage and cellularity of the newborn piglets.

MATERIALS and METHODS: Pregnant Landrace sows have been treated with 6 mg pST (Pitman-Moore, Inc.) injected daily i.m. for 15 days in different periods of gestation. The sows which were assumed to be pregnant were divided randomly into a control group and 3 test groups differing in the treatment period, which was between 10 and 24 (Group I), 50 and 64 (Group II) or 80 and 94 (Group III), respectively. After farrowing from each of the resulting 44 litters one piglet of representative birth weight was dissected. Several organs and hormone glands as well as *semitendinosus* muscles were prepared and weighed. Body composition was determined by chemical analysis of the autoclaved and homogenized piglet according to ENDER and HARTUNG (1987). *Semitendinosus* muscle sections were stained for acid-stable ATPase (preincubation at 4.2, GUTH and SAMAHA, 1970), or for haemalum-eosin for nuclei (ROMEIS, 1989). The number of nuclei and fibres was counted by an ocular grid. The number of type I fibres strongly reacting for acid-stable ATPase was counted in the clusters of the deep portion of the

muscle. The muscle cross area was obtained by planimetry. Nucleic acids and protein were analysed according to MUNRO and FLECK (1966), PETERSON (1977) and RICHARDS (1974), respectively. Differences between means were regarded as significant for $P < 0.05$ with student's t-test.

RESULTS and DISCUSSION: The treatment of pregnant sows with porcine somatotropin affected the development of the fetuses in a manner dependent on the gestational period of treatment as far as can be determined by means of the developmental stage at birth. The birthweight of piglets was enhanced by 4.8% in Group III compared to the controls and remained unchanged in Group I and II (table 1). No significant differences occurred in litterweight and littersize. In addition to the body weight significant differences in body composition were found only in Group III. The crude fat percentage slightly increased from 1.25% in control piglets to 1.4% ($P < 0.05$) due to water loss. Regarding the weights of organs and hormone glands the most striking differences again were found in Group III piglets, whose mothers were treated with pST during the late gestation (table 2). The weight of the lungs increased by 33% ($P < 0.05$). Stomach, liver, kidney, heart, thyroid gland and thymus showed an increase between 9 and 105 days. No changes were obtained in spleen, pancreas and adrenal gland weights.

On the macroscopic level no signs of an accelerated development of *semitendinosus* muscle were observed (table 3). There was no significant increase in muscle weight in any of the test groups, and in Group II a decrease actually seen ($P < 0.05$). On the other hand, several changes in muscle microstructure were evident. In Group III piglets, which appeared most mature at birth according to the above data, the number of nuclei per fibre ($P < 0.05$) and fibre area (mm^2) decreased. Accordingly, the DNA concentration showed a decreasing trend together with slightly elevated RNA concentrations and RNA/DNA values without higher protein levels. These data may indicate a precocious onset of fibre hypertrophy, which normally occurs at about 100 days of gestation. The increased number of muscle fibres/ mm^2 is rather an indication of the more compact arrangement of the fibres than of their smallness. Moreover, the muscles of Group III showed the highest fibre number per Type I cluster. In the distal portion of *semitendinosus* at first only primary fibres exhibit a positive reaction for active stable ATPase (Type I). Between 90 and 105 days of gestation the secondary fibres located

Table 1: EFFECTS OF pST TREATMENT OF PREGNANT SOWS ON THEIR NEWBORN PIGLETS (* $P < 0.05$)

	Control	Group I	Group II	Group III	SEM
Number of sows	12	11	13	8	
piglets	129	100	134	76	254
Birthweight (g)	1392	1364	1356	1459 *	3.4
Litterweight (kg)	15.0	12.4	14.0	13.9	2.6
Littersize	10.8	9.1	10.3	9.5	

Table 2: ORGAN AND HORMONE GLAND WEIGHTS OF NEWBORN PIGLETS IN RESPONSE TO pST TREATMENT OF THE SOWS DURING PREGNANCY (* $P < 0.05$)

	Control	Group I	Group II	Group III	SEM
No. of animals	11	11	13	8	1.22
Stomach-empty (g)	7.88	7.97	8.09	8.60	8.8
Liver (g)	47.2	48.7	43.8	52.7	0.46
Spleen (g)	1.69	1.55	1.74	1.59	2.4
Kidney (g)	11.4	11.5	11.8	12.7	1.6
Heart (g)	10.9	10.9	10.9	12.2	4.8
Lungs (g)	22.2	22.9	22.7	29.5*	0.53
Pancreas (g)	2.38	2.08	2.46	2.24	0.61
Thymus (g)	1.93	2.00	1.90	2.24	72
Adrenal gland (mg)	319	334	327	304	104
Thyroid gland (mg)	341	348	349	380	

Table 3: EFFECTS OF pST ADMINISTRATION TO PREGNANT SOWS ON CHARACTERISTICS OF SEMITENDINOSUS MUSCLE OF THE NEWBORN PIGLETS (* P < 0.05)

	Control	Group I	Group II	Group III	SEM
no. of animals	9 - 11	9 - 11	7 - 13	5 - 8	
muscle weight (g)	3.24	3.23	2.83 *	3.20	0.39
muscle length (mm)	43.4	44.5	43.8	47.3	4.1
cross sectional area (cm ²)	1.25	1.31	1.13	1.17	0.15
total muscle fibre number (x 10 ³)	302.7	383.9 *	298.7	315.8	48.7
nuclei/mm ²	2463	2992 *	2713	2821 *	442.6
nuclei/Fibre	2845	2956	2902	2645	280.9
primary fibre number/cluster	1.17	1.01 *	1.07	0.95 *	0.13
centrally nucleated primary fibres (%)	6.1	6.3	6.6	7.4 *	1.5
arrangement of animals)	2.08	0.83 *	1.04	1.24	2.61
loose	27	33	56	0	-
medium	18	11	11	42	-
compact	55	56	33	58	-

single Type I fibres exhibit a conversion from Type II to Type I ATPase histochemistry (BEERMANN et al., 1978). Type I fibre clusters are formed. Thus, a higher fibre number per cluster may indicate an advanced developmental stage of the muscle. In the semitendinosus muscles of Group I piglets, whose mothers were treated with pST between 10 and 24 days of gestation, a striking increase in muscle fibre number by 27% (i.e. 1000 fibres) was found. Accordingly, there is a slight tendency for more nuclei as well as DNA and RNA. The nuclei were distributed among more fibres as seen by the decrease of nuclei/fibre compared to controls (P<0.05) and, the fibres were smaller indicated by their lower number per unit area. During fetal myogenesis the central nuclei of the primary fibres migrate into a subsarcolemmal position causing in this way a decrease in centrally nucleated fibres. The muscle appeared more mature according to the lower number of primary fibres with centrally located nuclei (P<0.05). However, the number of Type I fibres and the fibre arrangement corresponded to control values. Thus, it seems possible that the lower number of centrally nucleated primary fibres is only due to the higher total fibre number and not to an advanced development of the muscle. - In Group II rather an impairment of muscle development might be concluded by lower muscle weight and protein content (P<0.05) as well as by the muscle loose arrangement of the fibres. This result should not be overestimated, for only since maternal growth hormone, insulin as well as somatomedins cannot penetrate the placenta and thus cannot directly exert a growth-promoting effect on the fetus, the fetal growth, most

Table 4: EFFECTS OF pST ADMINISTRATION TO PREGNANT SOWS ON PROTEIN AND NUCLEIC ACIDS OF SEMITENDINOSUS MUSCLE OF THE NEWBORN PIGLETS (* P < 0.05)

	Control	Group I	Group II	Group III	SEM
no. of animals					
protein (mg)	11	10	13	8	
DNA (µg/g)	291.5	295.9	234.6 *	243.2	43.6
RNA (µg/g)	937.6	1097.9	978.0	1052.5	298.4
RNA/DNA	957.5	1032.1	1019.0	910.0	227.2
	1.015	1.062	1.010	1.184	0.301

likely, was stimulated by exogenous pST in an indirect manner. The most obvious reason for the higher developmental stage of the piglets, whose mothers were treated with pST in late pregnancy, is the higher substrate availability, in particular of glucose. Maternal transplacental glucose is the primary energy-producing fuel in the fetus and, together with fetal insulin, is greatly responsible for fetal growth. Acceleration of fetal growth in mothers with *diabetes mellitus* and increased transplacental glucose supply to the fetus (hyperglycemia) resulting in hypersecretion of insulin is commonly recognized (DOOLEY and METZGER, 1980). Moreover, it has been suggested that maternal somatomedins stimulate placental growth and prolactin (hPL) production in humans and that hPL in turn stimulates placental somatomedin synthesis for direct release into the fetal circulation in support of fetal growth (e.g. ROBINSON et al., 1970). Furthermore, glucose itself is a determinant of IGF-1 secretion (BASSETT et al., 1990). SPENCER et al. (1983) found that insulin is able to enhance the bioassayable IGF-activity. The effect of maternal pST on the whole body of piglets only occurred after late gestational treatment. A compensatory effect after the withdrawal of the sows from pST in group I and II is imaginable; on the other hand the dependence on the provision of nutrients increases with advancing fetal age (DOOLEY and METZGER, 1980).

At birth the only remaining effect of the pST treatment in the early pregnancy was a significantly higher muscle fibre number in *semitendinosus* muscle. The muscle fibre number in turn is mainly determined by the number of myoblasts which are able to fuse and thus by the extent of their former multiplication during fetal myogenesis. It is imaginable, that exogenously increased maternal pST triggered an extraordinary growth factor-dependent proliferation of presumptive myoblasts in fetal muscle. The proliferative mitosis mainly occurred during the first part of the fetal growth (BEERMANN et al., 1978; SEIDEMANN et al., 1984) during treatment period I and physiological concentrations of insulin like growth factor 1 (IGF-1) have been shown to stimulate myoblast proliferation in vitro (e.g. FLORINI, 1987).

CONCLUSIONS: The administration of porcine somatotropin to pregnant sows during late gestation may affect the development of the fetuses on the whole resulting in an advanced physiological maturity of the newborn piglets. A treatment in the early pregnancy may induce the formation of more skeletal muscle fibres and, in this way enhance the capacity for postnatal muscle growth.

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