Arch. Tierz., Dummerstorf **43** (2000) 3, 223-230

Institute of Molecular Animal Breeding, Gene Center, Ludwig-Maximilian University, Munich, Germany

SABINE NEDBAL, NICOLA ZINK, HARALD LAHM, ANDREAS HOEFLICH and ECKHARD WOLF

Functional dissection of the insulin-like growth factor (IGF) system – prospects for animal breeding

Dedicated to Professor Dr. D. Simon on the occasion of his 70th birthday

Summary

Growth is a biological phenomenon that is subject to complex endo-, para-, and autocrine control mechanisms. In addition to insulin, thyroid hormones, sex steroids, and growth hormone (GH), components of the IGF system have been identified as key players in growth regulation. However, since altered growth is often associated with multiple changes in this complex regulatory network, the specific effects of individual components remain to be determined. Therefore, our lab focused on the functional dissection of the IGF system using transgenic mice and other animal models which are characterized by altered expression of individual members of this system. Here we review some of our findings identifying members of the IGF family as candidate genes which may affect important traits in livestock production.

Key Words: IGF, GH, mouse model, growth

Zusammenfassung

Titel der Arbeit: **Funktionale Entschlüsselung des Insulin-like Growth Factor (IGF) Systems - Bedeutung für die Tierzucht**

Wachstum ist ein biologisches Phänomen, das komplexen endo-, para- und autokrinen Kontrollmechanismen unterliegt. Neben Insulin, Schilddrüsenhormonen, Sexualsteroiden und dem Wachstumshormon (GH) spielen Komponenten des IGF Systems eine Schlüsselrolle in der Regulation von Wachstumsprozessen. Da in Situationen von verändertem Wachstum häufig viele Faktoren dieses Netzwerks in ihrer Expression reguliert sind, ist es schwer, einzelnen Komponenten eine spezifische Bedeutung beizumessen. Wir haben uns daher auf die funktionale Entschlüsselung des IGF Systems spezialisiert, wofür wir transgene Mäuse und andere Tiermodelle verwenden, die durch eine veränderte Expression einzelner Komponenten dieser Kaskade charakterisiert sind. Dieser Review faßt einige unserer Befunde zusammen, die Komponenten des IGF Systems als Kandidatengene für tierzüchterisch wichtige Merkmale identifizieren.

Schlüsselwörter: IGF, GH, Mausmodell, Wachstum

Introduction

The IGF system consists of two insulin-like growth factors (IGF-I and -II), two receptors and six binding proteins (IGFBP-1 to -6). IGFs are growth-promoting peptides, which show significant structural homology with insulin (RINDERKNECHT and HUMBEL, 1978a,b) and also biological effects similar to those of insulin (SOARES et al., 1985). They display growth-promoting effects, and stimulate cell growth and cell division (BASERGA et al., 1999).

Most of the biological effects of IGFs are mediated via the type I IGF receptor (IGF-I-

R), whereas the type II IGF receptor is - in addition to its role in trafficking of mannose-6-phosphate bearing lysosomal enzymes - responsible for the degradation of IGF-II. In addition, both IGFs can bind to the insulin receptor (ROTH and KIESS, 1994).

IGFs circulate in plasma complexed to six different binding proteins. These structurally related binding proteins are present in many tissues and biological fluids and are capable of transporting and modulating IGF actions. IGFBPs influence the bioavailability of IGFs to target tissues and their distribution in the extracellular environment (RAJARAM et al., 1997). They are also thought to have activities independent of IGFs such as direct receptor-mediated effects (KELLEY et al., 1996).

The IGFs are found in distinct molecular weight complexes (Fig. 1). Upon IGF binding, IGFBP-3 and -5 associate with an acid-labile subunit (ALS), forming ternary complexes of 150 kDa or 130 kDa, respectively (MARTIN and BAXTER, 1986; TWIGG and BAXTER, 1998). Part of the IGFs is also found in 50 kDa molecular weight complexes (SHIMASAKI et al., 1991) and only very little of the IGFs is present in an uncomplexed form (HASEGAWA et al., 1996). The half-life of the

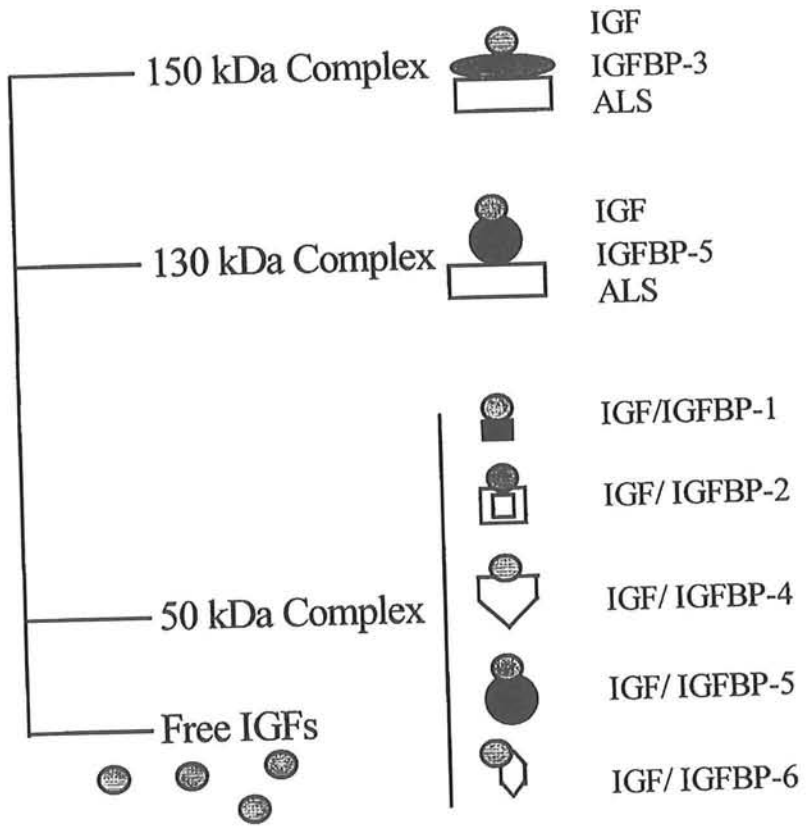

Fig. 1: IGFs (IGF-I or -II) in human serum (proposed model) (RAJARAM et al., 1997; TWIGG and BAXTER, 1998) (IGFs (IGF-I oder -II) im menschlichen Serum (vorgeschlagenes Modell))

IGFs is greatly enhanced by the IGFBPs (half-life of free IGF-I: 10-12 minutes; in the 50 kDa complex: 20-30 minutes; in the big complex: 12-15 hours; GULER et al., 1989).

Insulin, another anabolic peptide, is synthesized exclusively in the pancreatic islets of Langerhans, while IGFs are synthesized in multiple tissues throughout the body (SHIMASAKI et al., 1991). Insulin has no affinity to the IGFBPs (KELLEY et al., 1996) (Fig. 2).

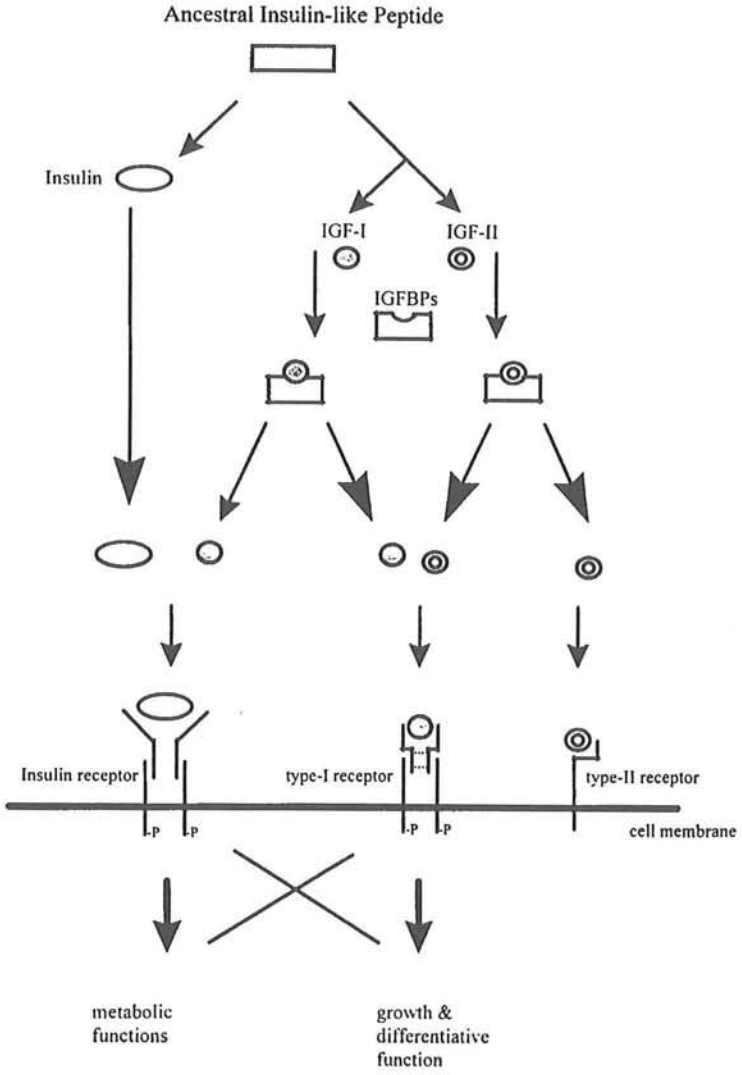

Fig. 2: Scheme of the IGF/IGFBP system (KELLEY et al., 1996) (Schema des IGF/IGFBP Systems)

Selection for body weight results in multiple changes in the IGF system

Factors affecting body growth, a major trait in animal breeding, have been investigated

in a plethora of studies. We evaluated the relationship between growth of mice selected for body weight and changes in the IGF system (HOEFLICH et al., 1998). Mice were generated by selection for high (H mice) or low eight-week body weight (L mice) over more than 50 generations and investigated for body and organ weights as well as serum IGF-I, insulin and IGFBP levels. Compared to control mice (C mice), the body weight of H mice was significantly increased whereas the body weight of L mice was significantly reduced (Table). In male mice, circulating levels of IGF-I were significantly reduced in the L line but significantly increased in the H line. Interestingly, in L mice we demonstrated a significantly reduced volume density of the somatotrophic cells in the anterior pituitary gland, suggesting a reduced secretory capacity for GH. This is in line with reduced serum levels of IGF-I and IGFBP-3, both of which are known to be regulated by GH. In contrast, hepatic IGFBP-2 mRNA expression and circulating IGFBP-2 levels were significantly increased in L mice, suggesting a potential negative function of this IGFBP for somatic growth.

Table
Characteristics of 8-month-old mice selected for high (H) or low (L) eight-week body weight as compared to controls (C) (HOEFLICH et al., 1998) (Charakteristika 8 Monate alter Mäuse, die auf hohes (H) oder niedriges (L) 8-Wochen-Gewicht selektiert wurden, im Vergleich zu Kontrolltieren (C) (HOEFLICH et al., 1998))

Parameter	Sex	L line mean (SD)	C line mean (SD)	H line mean (SD)	Comparison of groups L:C	L:H	C:H
Body weight (g)	M	21.7 (1.3)	44.8 (4.3)	63.1 (9.3)	*	*	*
	F	20.6 (1.4)	41.1 (3.5)	57.9 (4.9)	*	*	*
IGF-I (ng/ml)	M	285 (34)	447 (20)	600 (59)	*	*	*
	F	261 (44)	506 (109)	542 (59)	*	*	n.s.
IGFBP-2 (AU)	M	1355 (320)	405 (76)	329 (54)	**	*	n.s.
	F	785 (399)	529 (251)	262 (201)	*	*	n.s.

Significant differences are marked by asterisks (*P<0.05; **P<0.01); n.s. = not significant;
AU = optical density in arbitrary units

Transgenic models for clarifying the specific functions of IGFs

Consistent with the concept of IGF-I being an important regulator of somatic growth, transgenic mice overexpressing IGF-I under the control of the mouse metallothionein I (MT) promoter displayed increased body weight gain (MATHEWS et al., 1988). The tissues with the strongest increases in weight were spleen, pancreas, brain, kidney, and liver. Most importantly, the carcass weights were markedly increased (20%). The gastrointestinal tract represents another important target tissue of IGFs (READ et al., 1991). Accordingly, MT-IGF-I transgenic mice displayed enhanced growth of the small bowel, associated with increased villus height, crypt depth and greater numbers of crypt cell mitoses (OHNEDA et al., 1997), providing direct evidence for a strong involvement of the IGF system in growth and function of the gut.

Although IGF-II has been shown to be an important growth factor in fetal development (DECHIARA et al., 1990), the role of this peptide in postnatal growth physiology is less clear. Therefore, several transgenic models overexpressing IGF-II peri- and postnatally have been established (for review, see WOLF et al., 1998). In adult mice IGF-II serum levels decrease and represent about 10% of the IGF-I serum levels. Thus, an important role for somatic growth seems unlikely and - in contrast to IGF-I transgenic mice - no positive effects on overall body growth have been observed in IGF-II transgenic mice (WOLF et al., 1994, 1995a, 1998). Instead, distinct postive effects on the growth of specific organs – such as the adrenal glands in the case of PEPCK-IGF-II transgenic mice (WEBER et al., 1999) – have been observed.

Functional analysis of IGF receptors in knockout mice

IGFs and their receptors play important roles in embryonic development. Mice lacking a functional IGF-I receptor gene were born alive but died within minutes of respiratory failure. Body weight was reduced to approximately 45% and sections of these mice showed, beside hypoplasia of skeletal muscles, alterations of the nervous system, skin and of bone development (LIU et al., 1993).

Functional inactivation of the gene encoding the IGF-II receptor demonstrated that this gene is subject to paternal imprinting and that the IGF-II receptor is important for the degradation of IGF-II. Thus, IGF-II receptor deficiency in knockout mice resulted in increased levels of IGF-II, fetal overgrowth and multiple skeletal and organ abnormalities (WANG et al., 1994).

The roles of IGFBPs as modulators of IGF actions in vivo – lessons from transgenic mouse models

A number of transgenic mouse models have been created overexpressing IGFBP-1 under the control of different promoters. These studies demonstrated, that elevated levels of IGFBP-1 result in reduced somatic and specifically brain growth, impaired glucose tolerance and fasting hyperinsulinemia, and disturbed female fertility (for review, see SCHNEIDER et al., 2000).

To find out if increased IGFBP-2 serum levels – as observed in mice selected for low body weight - result in reduced body weight or represent only an epiphenomenon without intrinsic growth regulating effects, we produced transgenic mice in which a murine IGFBP-2 cDNA is expressed under the control of the CMV promoter (HOEFLICH et al., 1999). The mRNA expression of IGFBP-2 was investigated in several tissues (stomach, jejunum, colon, liver, adipose tissue, kidney, spleen, skeletal muscle, heart, lung, brain, salivary gland, adrenal gland) and could be detected as a transgene specific band (1.6 kb) in all investigated tissues except for the liver and as an endogenous band at 1.4 kb in spleen, colon, lung and liver. IGFBP-2 protein levels, determined by Western ligand blotting (as a band at 32 kDa) and Western immunoblotting (as a band at 34 kDa), were elevated in pancreas (as the organ with the highest expression) followed by stomach, heart, colon, and adipose tissue. In addition to these tissues, elevated levels of IGFBP-2 were found in kidney, small intestine, spleen,

salivary glands, lung, and adrenal glands. Further immunohistochemical investigations of the pancreas demonstrated IGFBP-2 overexpression specifically in the islets of Langerhans, the staining pattern suggesting β-cell specific IGFBP-2 expression. The determination of serum IGFBP-2 proved a 3-fold increase in IGFBP-2 transgenic mice. No effects on IGF-I or -II serum levels could be detected, likewise on serum levels of IGFBP-3 and IGFBP-4. Analysis of fasting and postprandial glucose and insulin levels showed a reduction of fasting glucose levels in IGFBP-2 transgenic mice with borderline significance. Most importantly, a significant reduction of body weight was seen in IGFBP-2 transgenic mice of both sexes starting from an age of 23 days. Comparing the absolute organ weights, only the spleen was significantly reduced in weight. In addition, the relative (to body weight) weights of kidney, lung, stomach and colon were significantly reduced. The reduction in body weight was mainly due to a significant reduction in carcass weight, both absolutely (13%) and as a function of body weight (12%). These data suggest that IGFBP-2 is a potent inhibitor of skeletal muscle growth.
To further clarify this point, we crossed CMV-IGFBP-2 transgenic mice with transgenic mice overexpressing bovine GH under the control of the rat PEPCK promoter. The latter model is characterized by 2- to 3-fold increased serum IGF-I levels (BLACKBURN et al. 1997a), and markedly increased body and organ growth (BLACKBURN et al., 1997b), including a pronounced hypertrophy of skeletal muscles (WOLF et al., 1995b). Coexpression of the CMV-IGFBP-2 transgene in PEPCK-bGH transgenic mice reduced the carcass weight by 27%, confirming our hypothesis that IGFBP-2 reduces muscle growth even in the context of elevated GH and IGF-I levels (A. HOEFLICH, S. NEDBAL and E. WOLF, unpublished).
Negative effects on smooth muscle cell growth have been demonstrated for IGFBP-4 (WANG et al., 1998) by use of an α-actin-IGFBP-4 fusion gene. In these mice IGFBP-4 was also hypothesized to act by negative interference with IGF-I, and IGFBP-4 was suggested as an antagonist of IGF-I action.

Conclusions

IGFs and IGFBPs are involved in plenty of aspects in the regulation of body and organ growth as well as in biology of reproduction. Transgenic and knockout mouse models are important tools to characterize specific functions of individual components of the IGF system (for review, see WOLF et al., 1998; SCHNEIDER et al., 2000) which is pivotal for a practical use in livestock production.

References

BASERGA, R.; PRISCO, M.; HONGO, A.:
IGFs and cell growth. In: The IGF System. Eds. ROSENFELD R.G. and ROBERTS JR. C.T. TOTOWA. NJ: Humana Press. Inc., (1999), 329-353

BLACKBURN, A.; DRESSENDÖRFER, R.A.; BLUM, W.F.; ERHARD, M.; BREM, G.; STRASBURGER, C.J.; WOLF, E.:
Interactions of insulin-like growth factor-II (IGF-II) and growth hormone (GH) in vivo: circulating levels of IGF-I and IGF-binding proteins in transgenic mice. Eur. J. Endocrinol. **137** (1997a), 701-708

BLACKBURN, A.; SCHMITT, A.; SCHMIDT, P.; WANKE, R.; HERMANNS, W.; BREM, G.; WOLF, E.:
Actions and interactions of growth hormone and insulin-like growth factor-II: body and organ growth of transgenic mice. Transgenic Res. **6** (1997b), 213-222

DECHIARA, T.M.; EFSTRATIADIS, A.; ROBERTSON, E.J.:
A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature **345** (1990), 78-80

GULER, H.P.; ZAPF, J.; SCHMID, C.; FROESCH, E.R.:
Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol. Copenh. **121** (1989), 753-758

HASEGAWA, Y.; HASEGAWA, T.; TAKADA, M.; TSUCHIYA, Y.:
Plasma free insulin-like growth factor I concentrations in growth hormone deficiency in children and adolescents. Eur. J. Endocrinol. **134** (1996), 184-189

HOEFLICH, A.; SCHMIDT, P.; FÖLL, J.; ROTTMANN, O.; WEBER, M.M.; KOLB, H.J.; PIRCHNER, F.; WOLF, E.:
Altered growth of mice divergently selected for body weight is associated with complex changes in the growth hormone/insulin-like growth factor system. Growth Horm. IGF Res. **8** (1998), 113-123

HOEFLICH, A.; WU, M.; MOHAN, S.; FÖLL, J.; WANKE, R.; FROEHLICH, T.; ARNOLD, G.J.; LAHM, H.; KOLB, H.J.; WOLF, E.:
Overexpression of insulin-like growth factor-binding protein-2 in transgenic mice reduces postnatal body weight gain. Endocrinology **140** (1999), 5488-5496

KELLEY, K.M.; OH, Y.M.; GARGOSKY, S.E.; GUCEV, Z.; MATSUMOTO, T.; HWA, V.; NG, L.; SIMPSON, D.M.; ROSENFELD, R.G.:
Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int. J. Biochem. Cell Biol. **28** (1996), 619-637

LIU, J.P.; BAKER, J.; PERKINS, A.S.; ROBERTSON, E.J.; EFSTRATIADIS, A.:
Mice carrying null mutations of the genes encoding insulin-like growth factor I (*Igf-1*) and type 1 IGF receptor (*Igf1r*). Cell **75** (1993), 59-72

MARTIN, J.L.; BAXTER, R.C.:
Insulin-like growth factor-binding protein from human plasma. Purification and characterization. J. Biol. Chem. **261** (1986), 8754-8760

MATHEWS, L.S.; HAMMER, R.E.; BEHRINGER, R.R.; D'ERCOLE, A.J.; BELL, G.I.; BRINSTER, R.L.; PALMITER, R.D.:
Growth enhancement of transgenic mice expressing human insulin-like growth factor I. Endocrinology **123** (1988), 2827-2833

OHNEDA, K.; ULSHEN, M.H.; FULLER, C.R.; D'ERCOLE, A.J.; LUND, P.K.:
Enhanced growth of small bowel in transgenic mice expressing human insulin-like growth factor I. Gastroenterology **112** (1997), 444-454

RAJARAM, S.; BAYLINK, D.J.; MOHAN, S.:
Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr. Rev. **18** (1997), 801-831

READ, L.C.; LEMMEY, A.B.; HOWARTH, G.S.; MARTIN, A.A.; TOMAS, F.M.; BALLARD, F.J:
The gastrointestinal tract is one of the most responsive target tissues for IGF-I and its potent analogs. In: Modern Concepts of the Insulin-like Growth Factors, Ed. SPENCER, E.M. Amsterdam: Elsevier Science Publishing Co., Inc., 1991, pp 225-234

RINDERKNECHT, E.; HUMBEL, R.E.:
Primary structure of human insulin-like growth factor II. FEBS Lett. **89** (1978a), 283-286

RINDERKNECHT, E.; HUMBEL, R.E.:
The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J. Biol. Chem. **253** (1978b), 2769-2776

ROTH, R.A.; KIESS, W.:
Insulin-like growth factor receptors: recent developments and new methodologies. Growth Reg. **4 Suppl. 1** (1994), 31-38

SCHNEIDER, M.R.; LAHM, H.; WU, M.; HOEFLICH, A.; WOLF, E.:
Transgenic mouse models for studying the functions of insulin-like growth factor-binding proteins. FASEB J. **14** (2000), in press

SHIMASAKI, S.; LING, N.:
Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5 and -6). Prog. Growth Factor Res. **3** (1991), 243-266

SOARES, M.B.; ISHII, D.N.; EFSTRATIADIS, A.:
Developmental and tissue-specific expression of a family of transcripts related to rat insulin-like growth factor II mRNA. Nucleic Acids Res. **13** (1985), 1119-1134

TWIGG, S.M.; BAXTER, R.C.:
Insulin-like growth factor (IGF)-binding protein 5 forms an alternative ternary complex with IGFs and the acid-labile subunit. J. Biol. Chem. **273** (1998), 6074-6079

WANG, J.; NIU, W.; WITTE, D.P.; CHERNAUSEK, S.D.; NIKIFOROV, Y.E.; CLEMENS, T.L.; SHARIFI, B.; STRAUCH, A.R.; FAGIN, J.A.:
Overexpression of insulin-like growth factor-binding protein-4 (IGFBP-4) in smooth muscle cells of transgenic mice through a smooth muscle α-actin-IGFBP-4 fusion gene induces smooth muscle hypoplasia. Endocrinology **139** (1998), 2605-2614

WANG, Z.Q.; FUNG, M.R.; BARLOW, D.P.; WAGNER, E.F.:
Regulation of embryonic growth and lysosomal targeting by the imprinted *Igf2/Mpr* gene. Nature **372** (1994), 464-467

WEBER, M.M.; FOTTNER, C.; SCHMIDT, P.; BRODOWSKI, K.M.H.; GITTNER, K.; LAHM, H.; ENGELHARDT, D.; WOLF, E.:
Postnatal overexpression of insulin-like growth factor II in transgenic mice is associated with adrenocortical hyperplasia and enhanced steroidogenesis. Endocrinology **140** (1999), 1537-1543

WOLF, E.; KRAMER, R.; BLUM, W.F.; FÖLL, J.; BREM,G.:
Consequences of postnatally elevated insulin-like growth factor- II in transgenic mice: endocrine changes and effects on body and organ growth. Endocrinology **135** (1994), 1877-1886

WOLF, E.; LAHM, H.; HOEFLICH, A.:
What is the function of IGF-II in postnatal life? Answers from transgenic mouse models. Growth Horm. IGF Res. **8** (1998), 185-193

WOLF, E.; RAPP, K.; BLUM, W.F.; KOLB, H.; BREM, G.:
Skeletal growth of transgenic mice with elevated levels of circulating insulin-like growth factor-II. Growth Regul. **5** (1995a), 177-183

WOLF, E.; WANKE, R.; SCHENCK, E.; HERMANNS, W.; BREM, G.:
Effects of growth hormone overproduction on grip strength of transgenic mice. Eur. J. Endocrinol. **133** (1995b), 735-740

Received: 2000-03-13

Accepted: 2000-04-10

Authors' addresses
SABINE NEDBAL, NICOLA ZINK, Dr. HARALD LAHM, Dr. ANDREAS HOEFLICH, Prof. Dr. ECKHARD WOLF
Institut für Molekulare Tierzucht, Genzentrum der Universität München
Feodor-Lynen-Str. 25
D-81377 München
Germany

E-mail: ewolf@lmb.uni-muenchen.de