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Utilization of Polypeptide Growth Factors to Improve Animal Reproductive Performance

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Ⅰ. INTRODUCTION

Animal reproductive performance could be affected by several regulatory factors, including nutritional, environmental, and genetic factors. Particularly, during the last half of this century, animal reproductive performance has been remarkably successful in improving the efficiency of livestock production. For some traits efficiency gains have been achieved with little or no knowledge of the genes underlying the traits. And, they have depended upon the phenotypic selection by statistical methods to estimate the genetic parameters of some reproductive traits. In spite of these successes, it is clear that recent advances in both developmental biology and molecular biology are set to revolutionize the practice of animal reproductive performance in the 21th century.

Hormones have been known to be the primary endocrine factors that regulate reproductive performance. Since epidermal growth factor was first identified from mouse submaxillary gland in 1960's, more than 50 types of growth factors have been characterized in several animal tissue or cell types. Compared to hormones, polypeptide growth factors in general show different biochemical structure but similar biological functions. There is ample evidence showing that growth factors regulate many aspects of physiological events in the reproductive organs and that they modulate them via acting either directly or indirectly as mediators of steroid or peptide hormones. In addition, they show tissue- and developmental stage-specific manners in many animal species (Lee, 1996; Ko, 1997). Several growth factors have been identified in reproductive organs, including epidermal growth factor (EGF), insulin-like growth factors (IGFs), transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and colony stimulating factor-1 (CSF-1).

Further researches on the biochemical and physiological aspects of growth factors are still needed. But, using advanced molecular and cellular biological techniques, the elucidation of roles of polypeptide growth factors in reproduction is thought to enhance the utilization of farm animals in the future. Therefore, this review describes the biochemical and physiological properties of some of growth factors expressed in reproductive organs with emphasis on IGFs and TGF-βs and includes some of recent data obtained from this laboratory. Also, the possible use of growth factors to improve animal reproductive performance in the future is summarized.

Ⅱ. GENERAL CHARACTERISTICS OF GROWTH FACTORS

Growth factors are a group of peptide regulatory molecules with certain characteristics:

(a) low molecular weight (usually less than 80 kDa)
(b) mostly glycosylated proteins
(c) local production in various tissue types
(d) specific cell-surface receptors with high affinity
(e) autocrine, paracrine, and/or endocrine modes of action
(f) ability to affect cellular proliferation and/or differentiation.

Therefore, polypeptide growth factors exert their mitogenic effect by interaction with specific cell surface receptors on responsive cells (Table 1). Many polypeptide growth factors form families of structurally related molecules that bind to common receptors. The binding of a growth factor to its receptor elicits a cascade of events, including protein phosphorylation, inositol-lipid breakdown, ion fluxes, and changes in gene expression.

Ⅲ. BIOLOGICAL PROPERTIES OF GROWTH FACTORS

Ovulation rate, litter size, and milk yield can be considered to be more important economic traits than others in farm animals. These traits have been known to be influenced mainly by genetic factors and hormones, but recent reports suggest these are also modulated by several growth factors (Johnson, 1993; Talhouk et al., 1996; Martal et al., 1997).

1. Insulin-like Growth Factors (IGF)

It consists of two factors : IGF-Ⅰ and IGF-Ⅱ. IGF-Ⅰ, also known as somatomedic C, is a basic polypeptide of 70 amino acids (AA) which is structurally related to proinsulin and more distantly to relaxin. IGF-Ⅱ, a 67 AA and slightly acidic peptide, exhibits ~70% homology with IGF-Ⅰ and is considered to be less growth hormone-dependent than IGF-Ⅰ. IGF-Ⅱ is found at high concentrations in fetal and neonatal sera of rodents and transcription of its gene is strongly down-regulated postnatally. This peptide growth factor classically has been considered a fetal growth factor.

The Type Ⅰ IGF receptor (IGF-Ⅰ-R) is a heterotetramer of $\alpha_2\beta_2$ subunits, which is structurally related to the insulin receptor, has intrinsic tyrosine kinase activity, and binds IGF-Ⅰ, IGF-Ⅱ, and insulin, albeit with differing affinities. The Type Ⅱ receptor (IGF-Ⅱ-R) is a monomeric protein (M_r of 250 kDa) identical to the

Table 1. General properties of polypeptide growth factors and their receptors found in the reproductive organs of animals

Growth factors	M_r (kDa)	Steroidal Regulation*	Characteristics of Receptors
IGF-Ⅰ	7.6	E/P	tyrosine kinase
IGF-Ⅱ	7.5	E/P	tyrosine kinase
EGF	6	E/P	tyrosine kinase
TGF-α	7.5	E	tyrosine kinase
TGF-β	25		serine/threonine kinase
acidic FGF	16	E/P	tyrosine kinase
basic FGF	17	E/P	tyrosine kinase
PDGF	28~32		tyrosine kinase
CSF-1	70~90	E/P	tyrosine kinase

* E(estrogen), P(progesterone)

cation-dependent mannose-6-phosphate receptor. This receptor does not exhibit tyrosine kinase activity and has no apparent affinity for insulin.

Circulating IGFs are invariably associated with binding proteins (IGFBPs) as an IGF-IGFBP complex. IGFBPs modulate (stimulate or inhibit) bioactivities of the IGFs. Recent interest in the IGFBPs has resulted in the identification and characterization of at least six distinct, though structurally-related, IGFBPs.

The expression of IGF system including ligands, receptors, and binding proteins has been intensively studied in reproductive organs in mammalian and avian species (Upton et al., 1998). Their expression was affected by hormones depending upon developmental status (Armstrong et al., 1998; Perks et al., 1999). Furthermore, IGFs alone or in combination with other growth factors were shown to regulate the blastocyst development of bovine oocytes or embryos *in vitro* (Palma et al., 1997; Rieger et al., 1998). Henricks et al. (1998) reported the presence of IGF-I-R on sperm, the presence of IGF-I in semen, and the ability of IGF-I to stimulate sperm motility. This finding provides evidence that the IGF system may be involved in the fertilization process. Collectively, these reports indicate that the IGF system is involved in a variety aspects of animal reproductive processes.

Lactation is the last part of reproduction process. Nutrients contained in mammary secretions support the development of neonates and growth factors have been known to be primary mitogenic factors for their intestinal growth. Among them, IGF-I present in colostrum or milk most reportedly is the major regulator for the development of mammary gland and neonates (Donovan et al., 1994). Especially, des-IGF-I, which is three amino acids shorter than IGF-I, showed the highest biological activity.

At present, a number of DNA markers are available for selecting breeds, so called marker-assisted selection (MAS) and their use has been quite efficient in term of time, labor, and budget. Recently, Fec^B gene and estrogen receptor gene have been shown to correlate with litter size in sheep and pigs, respectively (Montgomery et al., 1994; Rothchild et al., 1996). This prompted us to search for an endocrine marker instead of genetic marker which might be a useful tool to find any correlationship of physiological status with genetic traits of animals. Among several growth factor types, IGF-I as a possible candidate was chosen due to its wide range of bioactivity in reproductive processes. The preliminary data showed that serum IGF-I concentration tend to be different between two groups of gilts at all ages tested (Fig. 1). This result suggests that growth factors could be the possible physiological endocrine marker. Studies on other factors are in progress.

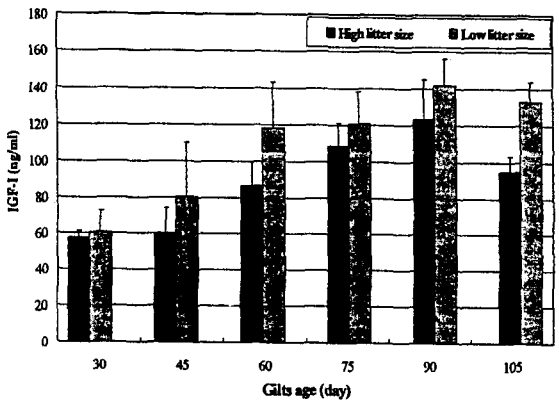

Fig. 1. Serum IGF-I concentrations of gilts from dam with history of either high or low litter sizes.

2. Epidermal Growth Factor (EGF)

EGF is a single chain, heat-stable, acidic polypeptide (53 AA, M_r of 6 kDa) that has strong mitogenic activity for various cell types both *in vivo* and *in vitro*. EGF was originally isolated from mouse submaxillary glands (Cohen, 1962) and subsequently from human urine (Gregory, 1975). Mature EGF is derived by proteolysis of the much larger preproEGF (1207 AA in human and 1217 AA in mouse) and the mature peptide is less structurally conserved among species than are the IGFs.

The EGF receptor (EGF-R) is a single-chain glycosylated polypeptide (1186 AA, M_r of 170 kDa) that exhibits tyrosine kinase activity and sequence-relatedness to the v-erb-B oncogene product.

Transforming growth factor-α (TGF-α) is a single-chain polypeptide of 50 AA (M_r of 7.5 kDa) derived from a 160 AA transmembrane precursor by protease cleavage. This protein exhibits 30% AA sequence homolgy with EGF and binds to EGF receptors, thus it belongs to EGF family.

Similar to IGFs, EGF also regulates proliferation and differentiation of many different cell types which are involved in various aspects of animal reproduction. EGF has been shown to have a positive effects on *in vitro* maturation and reported in follicular fluid at levels capable of stimulating meiosis in various species (Lonergan et al., 1996), which resulted in the wide use of this ligand in embryo culture system. EGF also showed mitogenic effects on trophoblast cells, indicating that EGF plays an important role in the implantation process by directly stimulating trophoblast development (Machida et al., 1995; O'Neill, 1997). And, Volentine et al. (1998) reported that EGF secreted from germinal disc stimulates follicular development in the chicken.

Lactoferrin is a member of the transferrin gene family. Its expression in the mouse uterus is regulated by estrogen and EGF (Teng, 1999), suggesting its possible roles in supporting ion transport during estrous cycles or pregnancy. Recently, due to the lack of lactoferrin in the infant formula, its mass production using transgenic cow has been reported (Han et al., 1996).

3. Hematopoietic Growth Factors

Their name was derived from the ability of such factors to stimulate the formation of colonies of neutrophilic granulocytes and monocyte-macrophages. Many cell types including fibroblasts, macrophages, endothelial cells, and T and B lymphocytes, can produce such factors in response to inductive stimuli.

An early observation of high levels of a factor in pregnant mouse uterus and fetal tissues that stimulated growth of bone marrow cells led to the subsequent identification of colony-stimulating factor-1 (CSF-1). CSF-1, also called monocyte-macrophage colony stimulating factor (M-CSF), is a homodimeric glycoprotein (M_r of 90 kDa) essential for proliferation, differentiation, and survival of mononuclear phogocytic cells. The CSF-1 receptor is a glycoprotein (M_r of 165 kDa) with intrinsic tyrosine kinase activity and is encoded by the *c-fms* proto-oncogene.

The observation of this cytokine in the bovine reproductive tract and of its role in the early development of embryo to the blastocyst stage leads to a possibility that it might be useful molecule for increasing blastocyst production rates in serum-free culture systems (de Moraes et al., 1999).

4. Heparin-binding Growth Factors (HBGFs)

The high affinity of these protein factors for

heparin, specifically heparan sulfate, found on cell surfaces and in extracellular structures, such as basement membranes, give rise to the name for this family of growth factors. Two well-characterized HBGFs are acidic fibroblast growth factor (aFGF) (pI 5.6, 140 AA) of a single-chain peptide synthesized in brain and other neural tisseus, and basic FGF (bFGF) (pI 9.6, 146 AA) of a peptide synthesized in pituitary, brain, adrenals, and ovary. Both FGFs exhibiting 55% amino acid sequence identity are multifunctional peptides implicated in cellular growth and differentiation, cell attachment, angiogenesis, and embryonic development. FGF receptors (FGF-R) that exhibit tyrosine kinase activity and that bind both aFGF and bFGF with differing affinities have been identified.

It was reported that mRNA and immunoreactive proteins for acidic and basic FGF and receptors were detected in the bovine reproductive tract (Gabler et al., 1997), suggesting its role in the process of implantation. Since proper regulation of angiogenesis and vascular permeability is essential for the physiological functioning of the female reproductive tract and embryology, they might be a potentially important intracellular regulator of endometrial, oviductal and embryonic function during early pregnancy in the cow. Furthermore, the bioactivity of these angiogenic growth factors was modulated by estrogens and progestins (Hyder and Stancel, 1999).

The mechanism of FGFs actions are complex and not well understood. Although high affinity cell surface receptors for FGFs exist, the FGF protein precursors do not have the usual signal peptide sequence necessary for secretion from cells. This has led to suggestions of unusual pathways for FGF secretion and of intracrine modes of action (Logan, 1990). Extracellular matrix (ECM)-degrading enzymes, such as heparanase or plasminogen activators, can potentially release the ECM-bound FGFs into the surrounding environment.

A type of proteoglycan, glycosaminoglycans (GAGs), is reportedly shown to stimulate cellular mitosis in combination with angiogenic growth factors including bFGF, TGF-β, and PDGF. Recently, this laboratory investigated to see if they could support the development of mouse embryos to blastocyst stage. As shown in Table 2, without any serum added, hyaluronic acid and chondroitin sulfate support the development of embryos significantly compared to control group. Also, the data show autocrine and/or paracrine mode of growth factors. More importantly, the result provides a possibility of GAG substitution with serum for *in vitro* embryo development, which might set for serum-free culture system.

5. Platelet-derived Growth Factor (PDGF)

PDGF is a basic (pI 9.8~10) glycoprotein acting as a mitogen and a chemotactic agent for various cell types. PDGF consists of either homo- or heterodimers of A (M_r of 16 kDa) and B (M_r of 14 kDa) chains, which exhibit 60% amino acid sequence homology. These PDGF dimers are remarkably resistant to heat and chemical denaturants, and the B-chain of PDGF is the product of the *c-sis* proto-oncogene.

PDGF initiates its biological effects by binding to its cell surface receptor which is a transmembrane glycoprotein (M_r of 170~180 kDa) with intrinsic protein tyrosine kinase activity.

6. Transforming Growth Factor-β (TGF-β)

TGF-β1 (originally described as TGF-β) is a disulfide-linked homodimer (M_r of 25 kDa, 112 AA/monomer) synthesized in an inactive form that is subsequently activated by changes in pH, addition of dissociating agents, incubation

Table 2. The effects of various GAGs on the development of mouse oocytes

Treatments	GAG (mg/ml)	No. of fertilized oocytes	Stage of development (%)			
			2-cell	4-cell	Morula	Blastocyst
Ham's F-10		31	31±0 (100%)	26±11.2 (83%)	24±14.2 (77%)	16±5.4 (54%)
Ham's F-10+FBS		49	47±6.7 (95%)	40±6.4 (81%)	37±1.5 (75%)	35±7.1 (63%)
Ham's F-10 + Hyaluronic acid	0.1	38	38±0 (100%)	34±10.1 (89%)	29±5.9 (76%)	24±7.6* (63%)
	0.5	48	44±9.5 (91%)	41±9.7 (85%)	36±6.3 (75%)	31±5.8* (64%)
	1.0	36	36±0 (100%)	31±9.3 (86%)	27±14.2 (75%)	23±6.9* (63%)
Ham's F-10 + Chondroitin sulfate	0.1	52	48±9.3 (92%)	45±4.2 (86%)	42±7.4 (80%)	29±1.8 (55%)
	0.5	45	42±4.8 (93%)	35±4.9 (77%)	35±4.9 (77%)	29±2.8* (64%)
	1.0	41	37±11.2 (90%)	33±9.0 (80%)	38±8.4 (73%)	23±5.1 (56%)

Values are shown as mean SE (percentage).
Values with * indicate significant effects ($P<0.05$).

with enzymes, or incubation with certain cell types. TGF-β1 belongs to a large family of both closely and distantly related proteins. TGF-β1 and 2 have been purified from mammalian cells and tissues, whereas TGF-β3, 4 and 5 were cloned by low stringency hybridization from Chinese hamster, chicken, and frog cDNA libraries, respectively. TGF-β1, 2 and 3 share most of their biological activities. Distantly related members of TGF-β superfamily include mammalian inhibins and activins, Mullerian Duct Inhibiting Factor, and Xenopus Vg1. TGF-β is a multifunctional polypeotide which can affect cell proliferation and differentiation, synthesis of extracellular matrix components, and morphogenesis during development (Choung et al., 1999).

Three types of specific, high-affinity TGF-β receptors are identified on various cell types: glycosylated type Ⅰ (M_r of 55~65 kDa) and type Ⅱ (M_r of 70~85 kDa), and high M_r proteoglycan type Ⅲ (M_r of 200~280 kDa). Both type Ⅰ and Ⅱ are transmembrane serine/threonine kinase receptors and must be present for TGF-β signal transduction; however, receptor type Ⅲ, also called β-glycan, has a large extracellular domain with a relatively small cytoplasmin domain that contains no obvious signaling motif.

TGF-β isoforms and their receptors exhibit cell type-specific patterns of expression. Information regarding the physiological function of TGF-β in the mammary secretions is limited. The presence of TGF-β1 and TGF-β2 were detected in colostrum and milk of cow and human (Tokuyama and Tokuyama, 1993) and thought to have immunoregulatory functions by stimula-

ting the production IgG and IgA from B lymphocytes (Donnet-Hughes et al., 1995). In contrast to the major role of TGF-β1 in most cell types, TGF-β2 seems to be the primary in the mammary secretions (Pakkanen, 1998).

Much evidence indicates that they play roles in various aspects of reproductive functions which include germ cell migration during embryogenesis and modulation of ovarian and testicular function in the adult. They show both overlapping and distinct spatial and temporal patterns of expression during different reproductive stages and differential hormonal stimulation in animals throughout development (Schimid et al., 1991; Gupta et al., 1996; Sanders and Wride, 1997; Choung et al., 1999).

Since TGF-β acts as an inhibitory factor for the proliferation and differentiation of most epithelial cells, it was hypothesized that TGF-β may inhibit sperm production in abnormal physiological status of human testis. Thus, the expression of TGF-β system in human testes with azoospermia has been studied using RT-PCR, showing that the testes with azoospermia showed higher levels of TGF-β isoforms than normal control group. With regards to receptor profiles, mRNA for type I was detectable but not for type II (Fig. 2) (Kim, 1999). This indicates that TGF-β isoforms are important regulators for spermatogenesis and that their signal transduction pathway is somewhat different from normal individuals. Further studies are in progress.

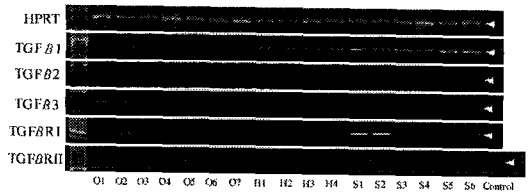

Fig. 2. The mRNA expression of TGF-β ligands and their receptors in human testes with azoospermia. The expression of TGF-β system in obstructive azoospermia group (Lane O1-O7), non-obstructive azoospermia group including hypozoospermia (Lane H1-H4) and Sertoli cell only syndrome (Lane S1-S6), and a positive control (Lane Control) was indicated by arrows at right. The sizes of bands were as follows: 226 bp (HPRT), 488 bp (TGF-β1), 439 bp (TGF-β2), 435 bp (TGF-β3), 658 bp (TGF-βR I), and 861 bp (TGF-βR II).

Ⅳ. APPLICATION OF GROWTH FACTORS FOR REPRODUCTION

Animal reproduction with high efficiency can be accomplished in concert with other important regulatory factors, such as genetic, environmental, nutritional, and endocrine/paracrine factors. Polypeptide growth factors acting via endocrine/paracrine modes regulate reproductive processes systemically or locally. However, effort to use growth factors efficiently for improving reproductive performance has hampered by limited knowledge on those factors. Thus, studies on biochemical and biological aspects of growth factors should be preceeded in order to fully understand the physiology of systemic regulation. Recent development of molecular biology techniques made it possible to some extent and studies using recombinant DNA are being accomplished widely.

In conjunction with molecular genetics, there could be two directions to improve valuable economic traits involved in animal reproduction. One is to inhibit (or prevent) the expression of negative factors with low performance and the other is to amplify the production of positive traits showing high efficiency. Several possible

ways of the growth factors to be used for improving animal reproduction is summarized.

· Selection of breeds showing high performance by growth factor profiles as MAS

· Production of transgenic bioreactor animals with growth factor gene

· Establishment of *in vitro* expression system for a mass production

· Establishment of serum-free culture system for producing embryos with high quality

· Elucidation of the correlation of growth factor action with steroid hormones

· Development of potential therapeutic agents for sterility

· Establishement of bioassays for early detecting diseases related to reproductive failure

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