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PREGNANCY PROTEINS: EARLY INDICATION OF PREGNANCY DIAGNOSIS IN FARM ANIMALS

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ABSTRACT

During gestation, many important molecular factors associated with embryo implantation and development occur within the female reproductive tract. The endometrium includes the muscosal lining of the uterus, which gives a convenient location for implantation and development of a fertilized egg and fetus. At present, the molecular cascades in the uterus endometrium during pregnancy periods in farm animals have not been elaborated completely. Many pregnancy proteins were expressed in the uterus endometrium during pregnancy. The expressions of these proteins involved in endometrium function and endometrium development from early to late gestation are associated with the regulation of endometrium development for maintaining pregnancy. The pregnancy proteins have key roles in tissue morphogenesis and growth. Growth promoters. Early pregnancy factor, Interferon, prolactin and Pregnancy associated Glycoproteins. Potential roles for pregnancy proteins have been identified in placental development and function, fetal growth, immunoregulation of pregnancy, embryo implantation and placental development. It is also necessary to consider the mechanisms controlling pregnancy from protein expression. This review focuses on pregnancy proteins as indication for early pregnancy diagnosis to highlight prospects for future research.

Key words: Pregnancy proteins, Expression, Function, Pregnancy diagnosis

1.INTRODUCTION

As a result of modern biotechnology, there are significant advances in the main concepts of animal reproduction. The discovery of chemical messengers (growth factors) and the presence of regulatory autocrine/paracrine systems have helped to unravel the secrets of reproduction.

Between the endometrium and the trophoectoderm, there are complex interactions. This leads to formation of different molecules which help during conception. These are harmonized by the conceptus which sends signals, which the uterus answers locally or contributes to modify the maternal endocrinology (Thibault C et al. 1998). Then, a new possibility opens to the researchers attentive with the development of tests for the diagnosis or the follow-up of gestation.

The placenta produces many molecules that play important role in implantation, fetal growth and maintenance of gestation (Wooding FBP, 1992). The fetal blood perfuses the placental or uterine boundary cells to carry oxygen and nutrients back to the fetus. The fetal blood also collects the hormones which are produced by placenta in the boundary cells. Most of these factors and hormones are proteins. The pregnancy protein expression study will help in the pregnancy diagnosis. The pregnancy diagnosis in domestic animals is important to differentiate diagnose of pregnancy with mummified fetus, comparative diagnosis in conjunction with other sero-diagnosis: brucellosis, leucosis, IBR, BVD, Visna Maedi, CAEV and while studying the embryonic or fetal mortality. It is now possible to detect more quickly of possible

placental dysfunctions occurring during gestation, affecting a more or less great part of the herd.

In this review, pregnancy proteins are briefly focused in relation to its expression timing and importance during pregnancy.

2. GROWTH PROMOTERS

Group of polypeptides are being produced by almost all the cells of the body. They carry on their biological activity via membrane specific receivers. They are intercellular regulators of the growth, differentiation and cellular mobility. They are directly implied in various processes such as cellular repair, immunizing protection and the ignition. Their mode of action is multiple: autocrine, paracrine and more rarely endocrine.

2.1. Insulin-Like Growth Factors (IGF) I and II

The insulin-like growth factor (IGF) system is a ubiquitous mitogenic system present in the uterine and/or conceptus environment of many species, including the cow, sheep, pig, horse and rodents. However, basic information concerning the role of the IGF system in regulating uterine physiology and conceptus development in cattle is lacking. The biological activity of the IGFs is modulated by a family of six IGF binding proteins (IGFBPs) whose own activity is regulated by the IGFBP proteases in cow. The integrity of IGF-I signalling is important for normal growth and brain development of fetus (Netchine I et al. 2011). IGFs, are important regulators of placental development and function (Forbes K and Westwood M, 2010).

In the cow, the expression of mRNA of the IGF I was observed in the embryo starting from the stage of 2 cells (Yoshida Y et al. 1998). It was more abundant in bovine embryos, which cleaved 27-30 hours after fecundation than in those which cleaved more tardily (Lonergan P, 2000). The absence of the form of gene *IGF I* in lines of knocked-out mouse is directly associated more than 95% of perinatal mortality like - reduction of more than 60% of the live weight of the born ones (Powell-Braxton L, 1993). Reduction of the expression of the IGF II in the mouse could be responsible for a reduction in not only the speed of growth of the blastocyst but also of the number of blastomeres (Rappolee DA et al. 1992).

2.2. Epidermal Growth Factor (EGF)

The EGF supports the compaction and the blastulation in the mouse (Wood SA and Kaye PL, 1989). Epidermal growth factor receptor (EGFR) signalling is essential for the proper fetal development of pancreatic islets and in the postnatal formation of an adequate beta cell mass (Hakonen E et al. 2011). Receivers for the EGF have already been identified in the bovine and ovine trophoblast from 13th and 15th days of gestation, respectively (Gharib-Hamrouche N, 1993; Kliem A, 1998).

2.3.Transforming Growth Factors (TGF) alpha and beta

In the ruminants, the biological activity induced by the receiver of the EGF can be due to its interaction with the TGF α, since TGF α and EGF borrow the same receivers (Han VK et al.1988). EGF and TGFα appear to be essential in early embryonic development in ruminant species. In the mouse and porcine embryo, EGF and TGFα are localized in trophoblast and are considered to have both autocrine and paracrine functions that are involved in elongation of the embryo (Dardik A et al. 1992; Vaughan TJ et al. 1992, Kliem et al. 1998). Thus, all the factors alluded to above may participate in the initial elongation of the bovine embryo (Blomberg LA et al. 2008).

TGFb1 and TGFb2 protein are found in the developing oocyte and embryo, (Ghiglieri C et al. 1995; Schmid et al., 1994) with TGFb2 being most abundant prior to fertilization and diminished in 4- and 8-cell embryos (Schmid et al., 1994). TGFb1 is expressed by uterine epithelium from early pregnancy (Tamada H et al., 1990) and during the implantation process is believed to enhance trophoblast attachment to the endometrium through eliciting production of the extracellular matrix component oncofetal fibronectin (Feinberg RF et al., 1994) and promoting adhesion of trophoblast cells to extracellular matrix (Irving JA and Lala PK, 1995).

The knocked-out mouse of *TGF β* gene seems to be directly responsible for a failure in the process of hematopoesis and differentiation of endothelial cells after the establishment, inducing a foetal mortality higher than 50% (Dickson MC, 1995).

2.4.Platelet-Derived Growth Factor (PDGF)

The expression of mRNA of the PDGF was observed starting from the stage 2 cells in the cow (Yoshida Y, 1998). The PDGF improves the development of the bovine embryo by supporting 8-16 cells passage. The PDGF could be one of the factors directly implied in the activation of the gene expression of bovine embryo during the 4th cellular cycle (Larson RC et al. 1992). Although reports of the role of PDGF in regulating fetal growth are limited (Forbes K and Westwood M, 2010), a recent study demonstrates that the maternal serum PDGFB level is enhanced in mothers suffering with gestational diabetes with macrosomic offsprings (Grissa O et al. 2010), and it has been reported that placental levels of PDGFRα are reduced in FGR placentas (Jarvenpaa J et al. 2007).

Platelet-derived growth factors (PDGFs) are soluble proteins that mediate intercellular signaling via receptor tyrosine kinases. The patterns of PDGF and PDGF receptor expression during embryogenesis are complex and dynamic and suggest that signaling can be autocrine or paracrine, depending on the particular tissue and the stage of development (Ataliotis P and Mercola M, 1997). PAF can mimic the effect of pregnancy, preventing the release of PGF2 alpha in response to exogenous oxytocin and, when administered into the uterine lumen, extending the life span of the corpus luteum. Thus, embryo-derived PAF appears to have an essential role in the establishment of pregnancy by acting as an autocrine growth factor for the embryo and by exerting a variety of effects on maternal physiology, including modulating maternal prostaglandin secretion and action (O'Neill C et al. 1990).

1.2. Fibroblast Growth Factors (FGF)

The FGF was detected for all the pre-implantation period in the cow and the ewe. The mRNA coding for the FGF was detected starting from the 8 cells stage in the mouse (Rappolee DA, 1994). However, the expression of bovine FGF was shown significantly higher at the beginning than at the end of the establishment of blastocyst (Watson AJ, 1992). In mouse, the FGF acts by stimulating the cellular division which gives origin to the embryonic and extra-embryonic cells. Interestingly, the effect on fetal growth was also accompanied by alterations in placental growth suggesting that FGF2 may exert its effects by influencing placental development (Forbes K and Westwood M, 2010).

3.EARLY PREGNANCY FACTOR (EPF)

There is existence of the EPF in maternal blood in mouse. (Morton H et al. 1974). The spermatozoon in the oocyte would produce a substance, the zygotine, which, would stimulate the production by the ovary carrying the yellow body of a factor called EPF (Cavanagh AC et al. 1982). The EPF was the resultant of the association of two elements: EPF A, secreted by the oviduct as well during the oestrus as during gestation, but inactive remainder a part from the latter, and the EPF B secreted by the ovary carrying yellow body in mouse (Cavanagh AC et al. 1984). The 70% of the sequence in amino acids of a molecule of “family EPF” are identical to the sequence of the chaperonine 10 in mouse. (Cavanagh AC and Morton H, 1994). The activity of EPF has been detected in the sera of mice, humans, and sheep from 6 to 24 hr after conceptive mating and persists for at least two thirds of the gestational period (Morton H et al. 1974; Morton H et al., 1977; Smart YC et al., 1982). Serum concentrations of the various EPF forms and their components vary from species to species and are dependent on gestational age (Clarke FM et al. 1980; Cavanagh AC et al. 1982; Rolfe BE et al. 1983).

Administration of monoclonal or polyclonal antibodies directed against EPF molecule would induce a reduction of the rate of gestation in mice (Athanasas-Platsis S et al. 1989). The embryos of immunized mice against EPF would probably not exceed the stage of 4-8 cells like result of an indirect effect on the maternal environment (Athanasas-Platsis S et al. 1991). Effect of the anti-EPF antibodies could be due to a maternal immunosuppression having like result and reduction of the expression of cytokines necessary to the embryonic development in mice (Morton H, 1992).

4. INTERFERON

Bovine interferon tau (boIFN-t) is one of the principal proteins secreted by the bovine conceptus from 16 to 25 days (Helmer SD, 1987). It has been showed that the “embryonic signals” emitted by the conceptus of the ruminants, initially called

trophoblastin or trophoblastic protein, constitute actually the same subclass of interferon, pertaining to the family of interferon alpha (Charpigny G, 1988).

In the ewe, the interferon tau (ovIFN-t) is produced between 13th to 21st days of gestation (Khan OA, 1998). The existence of an anti-luteolytic factor produced by the ovine conceptus leads to maintenance of the yellow body and the interruption of the oestrous cycle (Moor RM and Rowson LE, 1966). Interferon tau (IFN-t) regulates uterine *Mx* gene expression in ewes; however, the only known role for *Mx* gene is in the immune response to viral infection. It is hypothesized that *Mx* gene functions as a conceptus-induced component of the antiluteolytic mechanism and/or regulator of endometrial secretion or uterine remodeling during early pregnancy (Hicks BA et al. 2003).

Molecules belonging to the subclass of IFN-t were also identified on the level of the trophoblastic cells of the caprine conceptus from 14 to 17 days. However from the 18th day, when the establishment starts, the caprine IFN-t is not detected any more, suggesting that in this species, other factors are necessary from the 18th day of gestation to provide the luteotropic function (Guillomot M, 1998). These effects range from pregnancy recognition signaling in ruminants through IFN tau to effects on cellular functions of the uterus and uterine vasculature (Bazer FW et al., 2009).

5.PROLACTIN

This protein presents a primary structure very near to that of the growth hormone (GH) and placental lactogen hormone (PL). Members of the proliferin-related protein (PRL) family of genes have been given a variety of names, including placental lactogens (PLs), PRL-like proteins (PLPs), PRL-related proteins (PRPs), proliferin (PLF), and PLF-related protein (PLF-RP), whereas, members of the GH family are referred to as PLs, GH variant (GH-V), and chorionic somatomammotropins. In addition, the PRL23 can act via the traditional receiver (PRLR) to stimulate the angiogenesis in cow (Jabbour HN and Critchley HOD, 2001). The detection of the proliferin mRNA in a placental cDNA library also led to the discovery of another placental-specific hormone, designated PRP in cow. Expression of bovine PRPI (bPRP-I) in the placentome was examined during the preimplantation (days 17–19), implantation (days 20–25) and post-implantation (days 30–60) periods by immunohistochemistry, immunofluorescence and in situ hybridization.

During the preimplantation period, both bPRP-I mRNA and protein were undetectable in trophoblastic cells in cow. Both bPRP-I mRNA and protein appeared first at day 20 of gestation in trophoblastic binucleate cells and multinuclear cells that might migrate into the endometrium and fuse to epithelium in cow. These data indicate that bPRP-I may play a role before implantation and that bPRP-I may be an excellent marker for trophoblastic cell differentiation, as well as a candidate for pregnancy diagnosis (Yamada O et al. 2002).

6. PREGNANCY ASSOCIATED GLYCOPROTEINS

Several members of the family of PAGs are expressed in the cells of the trophoectoderm as of the stage of elongation of the hatched blastocyst in porcine species (Szafranska B and Panasiewicz G, 2002). Bovine PAG-1 has been used to provide sensitive tests for pregnancy in cattle and related ruminants (Mialon MM et al. 1993). Rodent and primate pregnancy-specific glycoprotein (PSG) gene families have expanded independently from a common ancestor and are expressed virtually exclusively in placental trophoblasts (McLellan AS et al. 2005). PAGs exert immuno-modulator role on the foeto-maternal level of the interface in cow (Roberts RM et al. 1996). During gestation of cow, the concentrations of PAG are detectable as early as from the 19th-22nd days after the conception, to reach concentrations from 3 to 6 ng/ml in the neighbourhoods of the 33rd-37th days of gestation (Perenyi Z, 2002). It means that this protein is detectable in the maternal peripheral circulation from approximately 98% of pregnant females starting from the 30th day of gestation in cow. During gestation, the concentrations of PAG rise initially gradually between 6th and the 35th weeks of gestation (4 ng/ml with 159 ng/ml), then rising more quickly between the 35th week and the last week of gestation (concentration of about 590 ± 1.550 ng/ml). The maximum values are reached 1

to 5 days before setting low (2462 ± 1.018 ng/ml) in bovine. The serum concentration of PAG decrease regularly and return below the threshold of detection (<0.2 ng/ml) between the 80th and the 120th days postpartum in bovine (Zoli AP et al. 1992).

The concentration of oPAG might be influenced by the fetal numbers and the sex of the fetus. Ewes carrying two fetuses had higher mean oPAG concentrations than those carrying a single fetus from week 12 of gestation to lambing. This difference was only significant at week 21. Also, ewes carrying male fetuses had oPAG concentrations higher than those carrying female fetuses at Weeks 19, 20 and 21 of gestation (Ranilla MJ et al. 1994).

Low PSG levels in the maternal circulation are associated with threatened abortions, intrauterine growth retardation and foetal hypoxia in mice and monkeys. The importance of PSGs for the maintenance of pregnancy is also underlined by the observation that the application of anti-PSG antibodies or vaccination with PSG induces abortion in mice and monkeys, respectively, and reduces the fertility of non-pregnant monkeys (McLellan AS et al. 2005).

7. CONCLUSIONS

Today, the experts have at their disposal different proportioning of proteins allowing the diagnosis of gestation. The pregnancy protein immunoassay has the advantages to detect pregnancy. As of today, one can retain the multiplicity of the signals, their specificity and the local and temporal character of their expression. A better knowledge of the early factors will make it possible to refine the methods of fecundation and culture of in vitro embryos. When used systematically in the farm, it would contribute to detect earlier some deteriorations of placental function affecting the fertility of the herd. The pregnancy proteins are important because; 1. These are essential for the early pregnancy diagnosis, 2. These can be used for preparation for pregnancy diagnosis kits, and 3. These pregnancy protein parameters can be used for diagnosis of stages of pregnancy diseases. A better knowledge of the factors expressed later will make it possible to define new protocols for the study of relations of mother-foetus during gestation, like for the study of embryonic mortality at the animal species of economic interest.

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