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Expression of activin receptors in the equine uteroplacental tissue: an immunohistochemical analysis

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Activin is secreted from equine uterine glands and plays important roles in establishment and maintenance of pregnancy in mares. This study aimed to localize activin receptors (ActRs) IA/B and IIA/B using immunohistochemistry in the uteroplacental tissues of seven pregnant Thoroughbred mares. At the time of tissue collection, the mares were at the following days of pregnancy: 88, 120, 161, 269, 290, 313, and 335 days. We fixed the uteroplacental tissues in 4% paraformaldehyde and obtained serial sections that were subsequently stained for analysis. All four isoforms of ActR were expressed in the uteroplacental tissues, including the endometrial epithelium, uterine glands, trophoblasts, and myometrium, throughout pregnancy. Our results suggested the potential role of activin in the uteroplacental tissues.

Key words: *activin, activin receptor, pregnant mare, uteroplacental tissues*

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The placenta is an essential endocrine organ that develops during pregnancy [13]. Although hormones such as estrogen, which stimulates fetal growth, and progesterone, which maintains uterine quiescence, are well established [1, 17], recent research has revealed the progression of another hormone, activin, in some animals, including horses [2, 9, 11].

Activin is a glycoprotein hormone that belongs to the transforming growth factor-β (TGF-β) family. It comprises two β-subunits and is divided into activin A (βA + βA), activin AB (βA + βB), and activin B (βB + βB) [7, 12]. In humans, the embryo and uteroplacental tissues produce and secrete activin A, which plays a critical role in maintaining pregnancy, including the preparation for implantation, embryo and placental development, and regulation of uterine immune responses by autocrine/paracrine actions [8, 9, 11, 19].

Activin receptors (ActRs) are transmembrane serine and threonine kinase receptors that primarily comprise four isoforms, ActR IA, IB, IIA, and IIB [7, 18, 23]. First, activin binds to a type II receptor, and a type I receptor is then recruited to form a receptor complex. Subsequently, the type I receptor is phosphorylated and activated, intracellular signals are transmitted, and activin effects are displayed [7, 18]. In recent years, some studies have suggested the possibility of activin A production and secretion in the equine uteroplacental tissues [2, 25, 26]. However, little information is available about the localization of ActRs in the uteroplacental tissues.

As loss of embryos and fetuses is a serious concern

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for the equine industry, establishing effective methods to minimize these concerns is imperative. In addition, maintaining a healthy uteroplacental environment is essential for successful pregnancy. Moreover, some studies have reported that activin has crucial roles in uteroplacental tissues. The present study aimed to investigate the localization of ActRs in the equine uteroplacental tissues and myometrium throughout pregnancy in Thoroughbred mares.

We examined seven pregnant Thoroughbred mares (age 9–20 years; Mean ± SD: 13.1 ± 3.7 years), were used in this study. All the mares were kept at The Hidaka Training and Research Center, Japan Racing Association (JRA), Hokkaido, Japan. The pregnancy periods of the mares at the time of sampling were 88, 120, 161, 269, 290, 313, and 335 days. We defined the last mating day as 0 days of pregnancy. Pregnant mares that died because of colic or were euthanized using an overdose of a mixture of thiopental sodium and suxamethonium chloride after intravenous administration of medetomidine (5 μg/kg) were dissected, and their uteroplacental tissues, including the myometrium, were immediately sampled, fixed in 4% paraformaldehyde, dehydrated in a graded series of ethanol, and embedded in paraffin. This study, including the sampling procedures and euthanasia, was conducted in accordance with the Animal Welfare and Ethics Committees at the Hidaka Training and Research Center, JRA.

We sectioned the tissue samples serially every 4 μm and placed them on slide glasses (MAS-GP type A, S9904, Matsunami Glass Ind., Ltd., Osaka, Japan). Subsequently, the sections were deparaffinized and rehydrated in xylene and decreasing ethanol series, respectively; endogenous peroxidase was deactivated by incubating the sections in 0.3% H_2O_2 in methanol at room temperature (RT). The antigen was retrieved in 1% citrate buffer (Vector® Antigen Unmasking Solution H-3300, Vector Laboratories, Inc., Burlingame, CA, U.S.A.) in distilled water using an autoclave, followed by washing in phosphate-buffered saline (PBS; 0.01 M, pH 7.4). Furthermore, sections were blocked with 2.5% Normal Horse Serum (ImmPRESS Reagent Kit, Vector Laboratories, Inc.) for 30 min at RT and washed with PBS. Sections were incubated with primary antibodies diluted in PBS with 0.5% Triton X-100 (PBS-Triton) overnight at 4°C. The primary antibodies used in this study were a goat polyclonal antibody to human ActR IA (1:320, ab115301, Vector Laboratories, Inc.), rabbit polyclonal antibody to human ActR I B (1:480, ab64813, Vector Laboratories, Inc.), mouse monoclonal antibody to human ActR IIA (1:160, ab76940, Vector Laboratories, Inc.), and rabbit monoclonal antibody to human ActR IIB (1:320, ab134082, Vector Laboratories, Inc.). The next day, sections were washed with PBS and incubated with anti-goat Ig (ImmPRESS Reagent Kit peroxidase, MP-7405, Vector Laboratories, Inc.), anti-rabbit Ig (ImmPRESS Reagent Kit peroxidase, MP-7401, Vector Laboratories, Inc.), or anti-mouse Ig (ImmPRESS Reagent Kit peroxidase, MP-7402, Vector Laboratories, Inc.) for 30 min at RT. Following washing with PBS, we the sections were colored using NovaRED (SK-4800, Vector Laboratories, Inc.), rewashed in PBS, and counterstained with Mayer's hematoxylin solution. In addition, all the sections were dehydrated using a graded series of ethanol, cleared in xylene, and coverslipped with a mounting agent (MGK-S, Matsunami Glass Ind., Ltd.). For negative control sections, PBS–Triton was substituted for primary antibodies, and each section was stained with hematoxylin and eosin (HE).

All four types of ActRs, ActR IA, IB, IIA, and IIB, were expressed in uteroplacental tissue structures, including the epithelial cells of the endometrium, epithelial cells of uterine glands, cells of trophoblast, and cells of smooth muscle in the myometrium, at 88 and 335 days of pregnancy (Fig. 1). In addition, a similar expression pattern of ActRs was observed in the other studied dates of pregnancy (data not shown). We observed no differences in staining intensity between the different days of pregnancy. Of note, negative control sections were not stained in the present study (Fig. 1).

In the present study, immunohistochemical results established the expression of ActRs, types IA, IB, IIA, and IIB, in the equine endometrial epithelium, uterine glands, trophoblasts, and myometrium. These findings suggest a potential role of activin in the regulation of placental formation and secretion of steroid hormone in pregnant mares. To the best of our knowledge, this study is the first to investigate the expression of ActRs in the reproductive organ of mares.

ActRs are expressed in the endometrial epithelium, uterine glands, and trophectoderm in ewes [10] and rats [5]. Based on these facts, expression of ActRs in endometrial epithelium, uterine glands, and trophoblasts was considered appropriate as results. As the expression pattern of ActRs did not differ during the period of 88–335 days of pregnancy in this study, the ligand concentration would be more important than the expression pattern of ActRs for activin effects.

In pregnant humans, activin A is produced in the uteroplacental tissues [8, 9, 15]. A previous studiy about pregnant horses has reported localization of βA subunits in the uterine luminal and glandular epithelium on day 25 of pregnancy [26]. In the present study, ActRs were detected in uterine gland epithelia, and it was suggested that activin A produced in the uterine gland epithelia may bind to ActRs in uterine glands and exhibit effects such as regulation of uterine gland development, as previously described [10].

In this study, we detected ActRs in the fetal and maternal placenta, and it was considered that activin A binds to ActRs and plays important roles in equine placental tissues, such as placentation, which occurs until day 150 of pregnancy

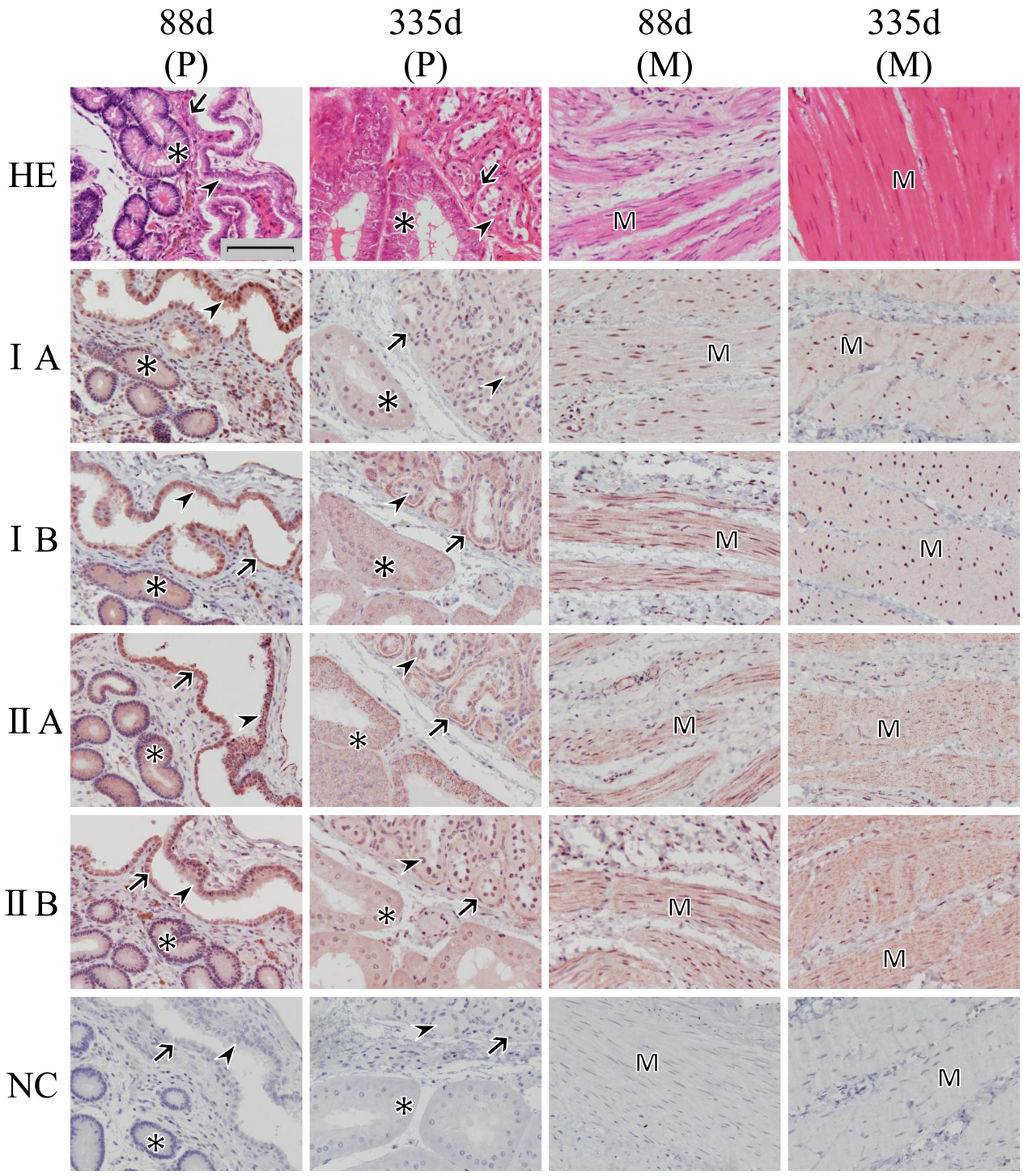

Fig. 1. Immunohistochemical staining of the uterine glands (*), uterine epithelium (➔), trophoblasts (➢), and myometrium (M) at 88 days (88d) and 335 days (335d) of pregnancy in Thoroughbred mares. Tissue sections were stained with antibodies against ActR IA, IB, IIA, and IIB. P, placental region; M, uterine smooth muscle cells in the myometrium; HE, hematoxylin–eosin staining; IA, ActR IA; IB, ActR IB; IA, ActR IIA; IIB, ActR IIB; and NC, negative control. All figures are at the same magnification, Scale bar, 100 μm.

[21]. This hypothesis is supported by a previous report that revealed, by Northern blot analysis, higher expression levels of activin βA subunit mRNA in equine uteroplacental tissues in the second trimester than in the third trimester of pregnancy [25]. However, the activin βA chain localizes in the uterine gland epithelium, not in the placenta including microcotyledons, trophoblasts, and maternal endometrial epithelial tissues at day 130–312 of pregnancy [2, 25]. Hence, as described previously [2], activin A may diffuse from uterine glands and bind ActRs in placental tissues.

Activin A stimulates the production and/or secretion of progesterone and estradiol in the human placenta [11]. The equine placenta supplies progesterone after approximately day 100 of pregnancy and estrogens during the second half of pregnancy [1]; this period is consistent with the period when the activin βA chain is expressed in the uterine glands [2, 25]. Based on these findings, we inferred that activin A from the uterine gland binds to ActRs in the placenta and stimulates hormonal production and/or secretion in horses.

Previous studies have raised the possibility that local activin A in humans [4] and blood activin A in rats [6] targeted the myometrium and inhibited its contraction [4]. Our study presented four types of ActRs in myometrial smooth muscle cells throughout pregnancy, suggesting that activin affects the myometrium during pregnancy and participates in the regulation of myometrial contraction, which is stimulated by oxytocin [14]. In a previous study, activin A inhibited oxytocin production in bovine luteinized granulosa cells [22]. In horses, oxytocin is produced in the endometrium [1, 3]; however, the same mechanism of inhibition of oxytocin production by activin could exist in the endometrium. Hence, activin may inhibit myometrial contraction by directly affecting the myometrium and indirectly inhibiting oxytocin production.

Activin is typically considered to be produced locally and show autocrine/paracrine actions [7]. In humans, activin A is considered to be a biologically active endocrine factor in late pregnancy [11]. Similarly, in horses, activin A may be active not only as an autocrine/paracrine factor but also as an endocrine factor in late pregnancy and may bind to ActRs in uteroplacental tissues revealed in this study. In addition to uterine grands, the ovary is known as a potential source of activin A in humans [20], swine [24], goats [23], and rats [16]. However, there are many unclear points concerning the relationships between ActR localization, activin origin, and activin effects. In the present study, we only performed immunohistochemistry. Further studies with other methods, such as PCR and ELISA targeting ActRs and the activin βA chain, are necessary to confirm our findings and the activin action mechanism.

In conclusion, this study established the expression of four types of ActRs, ActR IA, IB IIA, and IIB, in the endometrial epithelium, uterine glands, trophoblasts, and myometrium throughout equine pregnancy and suggested effects of activin in the uteroplacental tissues. Further studies are required to prevent equine abortion and premature birth.

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