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

Yuki KIMURA1,2, Motoki SASAKI1,2, Kenichi WATANABE1,3, Pramod DHAKAL2,4,6, Fumio SATO2,5, Kazuyoshi TAYA4 and Yasuo NAMBO1–3*

1Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan
2United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193, Japan
3Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary, Hokkaido 080-8555, Japan
4Department of Veterinary Medicine, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan
5Equine Science Division, Hidaka Training and Research Center, Japan Racing Association, Hokkaido 057-0171, Japan
6Present address: Animal Science Research Center, Division of Animal Science, University of Missouri, Columbia, MO 65211, U.S.A.

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
The antigen was retrieved in 1% citrate buffer (Vector® goat Ig (ImmPRESS Reagent Kit peroxidase, MP-7405, (1:320, ab134082, Vector Laboratories, Inc.). The next day, human ActR IIA (1:160, ab76940, Vector Laboratories, Inc.), mouse monoclonal antibody to polyclonal antibody to human ActR IB (1:480, ab64813, Vector Laboratories, Inc.), rabbit antibodies diluted in PBS with 0.5% Triton X-100 (PBS-Triton) 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% H2O2 in methanol at room temperature (RT). The antigen was retrieved in 1% citrate buffer (Vector® Antigen Unmasking Solution H-3300, Vector Laboratories, Inc.) 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 IB (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 trophoblasts. 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 study 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; IIA, ActR IIA; IIB, ActR IIB; and NC, negative control. All figures are at the same magnification, Scale bar, 100 µm.
contraction by directly affecting the myometrium and inhibited by activin in the endometrium. Hence, activin may inhibit myometrial 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 from the uterine gland and binds 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 granulomas, 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.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number 15K07735. The authors would like to thank the Hidaka Training and Research Center, JRA, Hokkaido, Japan, for the great technical advice for the experiments.

References

1. Allen, W.R. 2001. Fetomaternal interactions and influences during equine pregnancy. Reproduction 121: 513–527. [Medline] [CrossRef]
2. Arai, K.Y., Tanaka, Y., Taniyama, H., Tsunoda, N., Nambo, Y., Nagamine, N., Watanabe, G., and Taya, K. 2006. Expression of inhibins, activins, insulin-like growth factor-I and steroidogenic enzymes in the equine placenta. Domest. Anim. Endocrinol. 31: 19–34. [Medline] [CrossRef]
3. Aurich, C. 2011. Reproductive cycles of horses. Anim. Reprod. Sci. 124: 220–228. [Medline] [CrossRef]
4. Ciarmela, P., Wiater, E., and Vale, W. 2008. Activin-A in myometrium: characterization of the actions on myometrial cells. Endocrinology 149: 2506–2516. [Medline] [CrossRef]
5. Debiève, F., Hinck, L., Biard, J.M., Bernard, P., and Hubinont, C. 2006. Activin receptor expression and induction of apoptosis in rat blastocysts in vitro. Hum. Reprod. 21: 618–623. [Medline] [CrossRef]
6. Draper, L.B., Chong, H., Wang, E., and Woodruff, T.K. 1997. The uterine myometrium is a target for increased levels of activin A during pregnancy. Endocrinology 138: 3042–3046. [Medline] [CrossRef]
7. Ethier, J.F., and Findlay, J.K. 2001. Roles of activin and its signal transduction mechanisms in reproductive tissues. Reproduction 121: 667–675. [Medline] [CrossRef]
8. Florio, P., Cobellis, L., Luisi, S., Ciarmela, P., Severi, F.M., Bocchi, C., and Petraglia, F. 2001. Changes in inhibins and activin secretion in healthy and pathological pregnancies. Mol. Cell. Endocrinol. 180: 123–130. [Medline] [CrossRef]
9. Florio, P., Luisi, S., Ciarmela, P, Severi, F.M., Bocchi, C., and Petraglia, F. 2004. Inhibins and activins in pregnancy. Mol. Cell. Endocrinol. 225: 93–100. [Medline] [CrossRef]
10. Hayashi, K., Carpenter, K.D., Gray, C.A., and Spencer, T.E. 2003. The activin-follistatin system in the neonatal ovine uterus. Biol. Reprod. 69: 843–850. [Medline] [CrossRef]
11. Jones, R.L., Stoikos, C., Findlay, J.K., and Salamonsen, L.A. 2006. TGF-β superfamily expression and actions in the endometrium and placenta. Reproduction 132: 217–232. [Medline] [CrossRef]
12. Knight, P.G., and Glister, C. 2001. Potential local regulatory functions of inhibins, activins and follistatin in the ovary. Reproduction 121: 503–512. [Medline] [CrossRef]

13. Morresey, P.R. 2011. The placenta. pp. 84–95. In: Equine Reproduction, Vol. 1, 2nd ed. (McKinnon, A.O., Squires, E.L., Vaala, W.E., and Varner, D.D. eds.), Wiley-Backwell, Ames.

14. Morresey, P.R. 2011. Oxytocin, inhibin, activin, relaxin and prolactin. pp. 1679–1686. In: Equine Reproduction, Vol. 1, 2nd ed. (McKinnon, A. O., Squires, E. L., Vaala, W. E., Varner D. D. eds.), Wiley-Backwell, Ames.

15. Muttukrishna, S., Child, T.J., Groome, N.P., and Ledger, W.L. 1997. Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy. Hum. Reprod. 12: 1089–1093. [Medline] [CrossRef]

16. Ogawa, K., Kurohmaru, M., Shiota, K., Takahashi, M., Nishida, T., and Hayashi, Y. 1991. Histochemical localization of inhibin and activin alpha, beta A and beta B subunits in rat gonads. J. Vet. Med. Sci. 35: 207–212. [Medline] [CrossRef]

17. Ousey, J.C. 2004. Peripartal endocrinology in the mare and foetus. Reprod. Domest. Anim. 39: 222–231. [Medline] [CrossRef]

18. Pangas, S.A., and Woodruff, T.K. 2000. Activin signal transduction pathways. Trends Endocrinol. Metab. 11: 309–314. [Medline] [CrossRef]

19. Rabinovici, J., Goldsmith, P.C., Librach, C.L., and Jaffe, R.B. 1992. Localization and regulation of the activin-A dimer in human placental cells. J. Clin. Endocrinol. Metab. 75: 571–576. [Medline]

20. Roberts, V.J., Barth, S., el-Roeiy, A., and Yen, S.S. 1993. Expression of inhibin/activin subunits and follistatin messenger ribonucleic acids and proteins in ovarian follicles and the corpus luteum during the human menstrual cycle. J. Clin. Endocrinol. Metab. 77: 1402–1410. [Medline]

21. Samuel, C.A., Allen, W.R., and Steven, D.H. 1975. Ultrastructural development of the equine placenta. J. Reprod. Fertil. Suppl. 23: 575–578. [Medline]

22. Shukovski, L., and Findlay, J.K. 1990. Activin-A inhibits oxytocin and progesterone production by preovulatory bovine granulosa cells in vitro. Endocrinology 126: 2222–2224. [Medline] [CrossRef]

23. Silva, J.R.V., van den Hurk, R., van Tol, H.T.A., Roelen, B.A.J., and Figueiredo, J.R. 2004. Gene expression and protein localisation for activin-A, follistatin and activin receptors in goat ovaries. J. Endocrinol. 183: 405–415. [Medline] [CrossRef]

24. van den Hurk, R., and Van de Pavert, S.A. 2001. Localization of an activin/activin receptor system in the porcine ovary. Mol. Reprod. Dev. 60: 463–471. [Medline] [CrossRef]

25. Yamanouchi, K., Hirasawa, K., Hasegawa, T., Ikeda, A., Chang, K.T., Matsuyama, S., Nishihara, M., Miyazawa, K., Sawasaki, T., Tojo, H., Tachi, C., and Takahashi, M. 1997. Equine inhibin/activin β A-subunit mRNA is expressed in the endometrial gland, but not in the trophoblast, during pregnancy. Mol. Reprod. Dev. 47: 363–369. [Medline] [CrossRef]