Research Journal of Dairy Sciences 6 (2): 15-18, 2012
ISSN: 1993-5277

Levels of Prostaglandins and Angiotensin in Retained Placenta of Cattle

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Abstract: The aim of this study is to explore the endocrine mechanisms of placenta retention. Endocrine factors play a role in the manifestation of placenta retention in dairy cows. Prostaglandin E causes relaxation of smooth muscle in the non-pregnant uterus and uterus placenta angiotensin can regulate placenta prolactin release, regulate steroid and prostaglandin synthesis, modulate placenta blood pressure, stimulate uterine smooth muscle contraction. To investigate the relationship between placenta retention and placental prostaglandins and angiotensin, 10 cows did not release the fetal membranes within 12 h after parturition and served as the retained placenta group and 10 cows in that group which released their fetal membranes within 12 h, served as the health group. Levels of these endocrine factors in retained placenta were measured with ELISA and compared with those in normal placentas. Results showed that retained placenta had significantly lower levels of prostaglandins and angiotensin II ($p<0.01$). This observation suggests that the lower levels of Prostaglandins (PGE_2) and angiotensin II may participate in the development of placenta retention.

Key words: Placenta retention, cattle, fetal membrane, prostaglandins, angiotensin, China

INTRODUCTION

Prostaglandin E (PGE) causes relaxation of smooth muscle in the non-pregnant uterus while Prostaglandin F (PGF) works opposite. Corticotropin Releasing Hormone (CRH) stimulates the expression of PGHS 2 synthesis through the CRE sequence. CRH causes uterus contraction by decreasing prostaglandin dehydrogenase activity, promoting the release of prostaglandin D in a dose dependent manner from placenta, amniotic and chorionic membrane. It has been proposed that CRH receptor subtype may maintain the resting state by cAMP or suppressing myometrium PGE_2 synthesis. At full term, these subtypes may have been repressed and CRH displays a central role in uterine contraction for placenta discharge (Grammatopoulos and Hillhouse, 1999).

CRH and Oxytocin (OT) synergistically stimulate oxytocin receptor synthesis by myometrium, enhancing uterine contraction (Joseph *et al*., 1999). Estrogen affects the expression of Contraction Associated Proteins (CAP), further boost reactivity of late term uterus in primates. Progesterone maintains the resting state of uterus by blocking CAP expression, binding with oxytocin receptor and inducing high PGDH expression and limiting chorionic PG synthesis. Decrease in maternal plasma progesterone/oxytocin ratio is beneficial to the secretion of PG and contraction of myometium which initiating labor, separating and expelling placenta (Thomson, 1998; Joseph *et al*., 1999; Allen, 2001; Takagi *et al*., 2002). PGs and oxytocin are commonly subscribed in veterinary to prevent or treat effectively placenta retention.

Angiotensins (Ang) include Ang I-III. Blood loss or decreased kidney blood flow stimulates renin secretion from juxtaglomerular apparatus. Upon entering blood stream, rennin stimulates the conversion of hepatic pre-angiotensin to Ang I (10 amino acid residues). When Ang I passes the lung it is converted to Ang II (8 amino acid residues). A proportion of AngII is converted to Ang III (7 amino acid residues) by enzymes in plasma and tissues. Ang I stimulates epinephrine secretion from adrenal medulla. Epinephrine does not directly act on blood vessel contraction. Ang II increases the tension of arterioles resulting in the increase of blood pressure. In addition, Ang II stimulates adrenal cortex secretion of aldosterone which preserves Na, water and releases K in turn this leads to increase in blood volume and blood pressure. Ang II is the major active agent in rennin angiotensin system, exerting its biological function through binding with specific Angiotensin Type Receptor (ATR). In summary, uterus-placenta angiotensin has the following functions: stimulating stroma cell decidulization, regulating placenta prolactin release, regulating steroid and protaglandin synthesis, modulate placenta blood pressure, stimulating uterine smooth muscle contraction and regulating vascular permeability (Qing-Bin *et al*., 2000; Li-na *et al*., 2005; Feng-hua *et al*., 2008).

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Placenta retention is one of the common diseases after delivery. This disease has lead to a great loss in dairy industry. The cause of placenta retention is very complex (Zhou and Wang, 2008). Many plasma reproduction hormones have been shown to be closely related to the manifestation of placenta retention (Zhou and Wang, 2008; Liu *et al*., 2001; Zhixi *et al*., 1994). However, there are scarce studies on the relationship between placenta retention and plasma PGE_2 and Ang II. Here, researchers show that retained placenta tissues have significantly lower Ang II and PGE_2. This observation is important for the understanding of the cause of placenta retention.

MATERIALS AND METHODS

Animals and diagnosis: Placenta tissues were collected from Cows the Dairy Barn from 2009-2012 at the Agricultural University of Hebei. None of the cows from which placenta were collected had >4 L. Retained placentas were collected from 10 cows. Normal placentas were collected from healthy cows with closely matched age, number of birth and labor time. Animals were raised under ordinary condition without history of steroid treatment.

Criteria for healthy cows were medium or better nourished, smooth and natural delivery, placenta release within 12 h, calf healthy, no complications. Criteria for placenta retention were natural birth, fetal membrane retained or partially retained for at least 12 h.

Sample collection and treatment

Healthy control: Fetal membrane lobe was selected for the test. Under natural condition when fetal membrane was completely discharged, the freshest part was dissected, stored in plastic bag at -20°C.

Retained placenta: When placenta retention was diagnosed, placenta membrane was isolated surgically from uterus. Freshly separated lobe was selected, wrapped in plastic bag and stored at -20°C until analysis.

Reagent and equipment: ELISA kits for PGE_2 Ang II were manufactured by ADL (USA). Here 680 ELISA reader was purchased from Bio-Rad. MIKRO 220R refrigerated bench top centrifuge was purchased from Hettich (Germany).

Major clinic observations: Occurrence of placenta retention and endometritis monitored. Times of fetal membrane separation, lochia clearance, first estrus and milk yield were recorded.

Hormone level measurement: Hormone levels were measured with ELISA Method. Frozen placenta tissues were cooled down in liquid nitrogen for 1 h and homogenized with mortar and pistol. Homogenate (5 mL) was centrifuged at 10,000 rpm for 3 min and supernatant was collected for PGE_2 and Ang II measurement according to the protocol from the manufacturer. OD values were read with ELISA reader at 450 nm.

Data analysis: Using the standards from the kit, standard curve and equation were established for PGE_2 and Ang II concentrations. Data were examined with t-test.

RESULTS AND DISCUSSION

Clinic observations: Cows of healthy control were normal in vaginal discharge and became pregnant within 90 days (3 estrus cycles) postpartum. Within the same period, 3 cows with retained placenta became pregnant. Among the 7 cows that did not conceive within 90 days, 7 cows had uteritis and were treated with antibiotics. The one did not show obvious symptom of uteritis was suspected to have hidden inflammation in reproduction system. The placenta retention group had significantly lower pregnancy rate (30%) than the healthy control group ($p<0.01$, Table 1).

PGE_2 content in placenta tissue: PGE_2 concentration in retained placenta was markedly lower than in healthy control ($p<0.01$). Results indicate reduced PGE_2 concentration is closely associated with placenta retention (Table 2).

Ang II concentration changes in placenta: Retained placentas had significantly lower Ang II concentration than normal placenta ($p<0.01$). These results demonstrate that low Ang II concentration is closely related to placenta retention (Table 3).

Because of the special placenta structure, cattle expel fetal membrane much later, compared to other livestock. Cattle have higher placenta retention rate. The expulsion of placenta in cattle proceeds in two steps, separation and expulsion. Placenta separation starts at the end of pregnancy. Under the influence of hormone, placental cells at the junction between the maternal tissue starts to change and the cell layers become thinner and the

Table 1: Clinical observation in post partum cows

Groups	No.	Rate of placenta retention (%)	Rate of uteritis (%)	Pregnancy with 90 days	Pregnancy rate (%)
Control	10	0	0	10	100
Placenta retention	10	100	70	3	30**

*Denotes $p<0.05$ and **denotes $p<0.01$. Symbols are the same there after

Table 2: Levels of placenta prostaglandins (pg mL^{-1})

Groups	1	2	3	4	5	6	7	8	9	10	x±SD
Control	5.47	6.23	5.57	6.12	5.85	4.62	5.56	5.71	5.21	5.42	5.58±0.46
Retained placenta	4.43	5.18	4.90	5.14	5.35	5.09	4.56	4.02	4.53	4.18	4.74±0.46**

Table 3: Placenta angiotensin II levels (pg mL^{-1})

Groups	1	2	3	4	5	6	7	8	9	10	x±SD
Control	3.26	2.94	3.64	2.89	3.82	2.94	3.99	2.62	3.21	3.09	3.24±0.44
Placenta retention	1.96	1.61	2.94	2.71	2.09	2.29	1.97	1.86	2.02	2.95	2.24±0.47**

*Denotes $p<0.05$ and **denotes $p<0.01$. Symbols are the same there after

connective tissue become collagenized. At the same time, a large number of binucleated trophoblast giant cells appear until parturition. At the beginning of labor, local estrogen and prostaglandins increase in placental tissues and cells infiltrate. The contraction of uterus alternative cut off blood to uterus and villi which lead to further changes in the junction of placental tissue. After the delivery, umbilical cord is cut off and placenta blood circulation stops. Blood vessels in uterus and villus are reduced greatly. Subsequently, villi are disconnected from uterine navel. And finally, placenta is separated from uterus. From the process of uterine contraction and fetal membrane expulsion, it is conceivable that endocrine factors and uterine contractions are essential. Any factor that interferes with placenta separation or uterine contraction is potential cause of placenta retention.

PGE and PGF, respectively relax and contracts smooth muscle in non-pregnant uterus. But both promote smooth muscle contraction in pregnant uterus. Smooth muscle cells and endothelia have oxytocin receptor. The binding of oxytocin to its receptor causes the increase in intracellular Ca^{2+} stimulating contraction. The binding of oxytocin with receptors on the endothelia induces endothelia to produce prostaglandins. Through paracrining, prostaglandins stimulate smooth muscle contraction. Prostaglandins are widely used in human clinic to induce labor, late abortion, treat stillbirth and cervical dilation. They have also been used in natural abortion and incomplete abortion (Liu *et al*., 2001; Yu *et al*., 2006). In veterinarian clinic, prostaglandins are commonly used to treat reproduction and obstetric disease with considerable benefit. Studies showed that uterine contraction is closely related to prostaglandin concentration and receptor distribution. There are few reports that describe the relationship between prostaglandins and placenta retention. The current study demonstrated that retained cattle placenta (4.74±0.46 pg mL^{-1}) has significant lower PGE_2 compared with normal controls (5.58±0.46 pg mL) ($p<0.01$). This result did not completely agree with that of previous study in which showed the PGE_2 content in the RFM group was significantly higher than that in the control group after 6 h parturition (Takagi *et al*., 2002). In their study, the results about PGs concentrations in placental tissues were all from RFM cows in which parturition was induced by PGF2 injection. Therefore, it is obvious that researchers need further investigations to clarify the differences between the placental samples derived from spontaneous and induced parturition.

Ang II exerts its biological function through binding with ATR which is expressed at high level in placental vascular smooth muscle cells, capillary endothelium, trophoblast, syncytiotrophoblasts. Activated ATR is coupled with Gi/o protein resulting in suppression of AMP cyclase which in turn leads to the reduction of cAMP synthesis and finally vascular contraction. Activated ATR causes the opening of calcium channel, allowing influx of extracellular calcium, promoting aldosterone synthesis and finally participating in vascular contraction (Cooper *et al*., 1999). *In vitro* test shows that Ang II stimulates PGE_2 release and alkaline phosphatase activity (Petit *et al*., 1989). Ang II stimulates uterine and placenta local hormone synthesis and secretion, the release of oxytocin and β-1 glycoprotein from trophoblast tissue (Schauser *et al*., 1998) and uterine PGE_2 and PGI synthesis (Hagemann *et al*., 1994.). Ang II increases vascular permeability and angiogenesis (Schauser *et al*., 1998; Hagemann *et al*., 1994) regulates blood flow through placenta. During pregnancy, uterus placenta vascular resistance and blood flow are regulated in a very complex way. Ang II maintains placental blood pressure and blood supply. Low dose of Ang II increases uterine blood flow by promoting vascular relaxing agents. High dose of Ang II reduces uterus placenta blood flow. Application of suppressor of Ang II converting enzyme causes the reduction of uterus placenta blood flow even resulting the death of fetus in rabbit (Hagemann *et al*., 1994). Experiment shows that Ang II stimulates uterine smooth muscle contraction, increases intracellular calcium concentration and leads to phosphorylation of myosin light chain (Qing-Bin *et al*., 2000). Isochemia develops due to blood vessel contraction. This is one of the major factors that initiate fetal membrane expeuslsion. However, the relationship between placenta Ang II concentration and fetal membrane expulsion has been rarely reported. The results shows retained placentas (2.24±0.47 pg mL^{-1})

have lower Ang II concentration, compared with placentas normally (3.24±0.44 pg mL^{-1}) expelled. The concentration difference was significant. The result suggests that lowered placenta Ang II concentration is closely related to placenta retention.

CONCLUSION

Results from the current study conclude that low prostaglandin and Angiotensin II concentrations are closely related to placenta retention in dairy cows. The reduction of prostaglandin and angiotensin contents may be an important cause for placenta retention.

ACKNOWLEDGEMENT

This study was supported by Natural Science Foundation of Hebei Province, China (No. C2010000659).

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