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Possible role of the nuclear factor kappa beta (NF-κB) in the development of retained placenta

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Abstract
Abundant evidence suggests that NF-κB has a role in the physiology and pathophysiology of labour. The current study was undertaken to test the hypothesis that NF-κB may play a role in the pathogenesis of retained placenta (RP) in buffalo. Results of the present study revealed that there is a significant decrease in the serum level of NF-κB in buffalos with retained placenta (BRP) compared with its level in buffalos with normal placental release (NPR). Significant decreases in the serum levels of TNF-α and NO were also observed in BRP. Meanwhile, PGE2 level was significantly increased in the serum of BRP while the serum level of oxytocin (OT) was not significantly changed in both groups. The possible effect of the decreased activity of NF-κB in reducing myometrial contraction and in causing immunosuppression as two basic mechanisms in the etiology of RP was discussed.

Keywords: NF-κB, retained placenta, buffalo

Introduction

Retention of fetal membranes in cows is a very serious disorder which occurs in the last phase of parturition. It has a significant negative influence on health, welfare, milk productivity and further reproduction in the postpartum (Laven & Peters, 1996) [23]. Understanding the physiological events regulating the separation and expulsion of fetal membranes is a prerequisite for the treatment and prevention of retained fetal membranes in the bovine.

Retained placenta (RP) constitutes a syndrome, in that it is the outcome of multiple etiologies. Nuclear factor-kappa B (NF-κB) is a transcription factor family classically associated with inflammation, and is activated in response to infection and pro-inflammatory cytokines. This transcription factor has been proposed to have a pivotal role in parturition (Lindström & Bennett, 2005; Kim *et al* ., 2015) [25, 19]. Many pro-inflammatory and labour-associated genes are regulated by NF-κB, and aberrant NF-κB activity underlies a number of inflammation-related disorders (Lindström & Bennett, 2005) [25]. Human labour, both at term and preterm, is preceded by NF-κB-mediated inflammatory activation within the uterus leading to myometrial activation, fetal membrane remodelling and cervical ripening (Kim *et al* ., 2015) [19]. Thus, aberrant NF-κB activity is expected to be one of the mechanisms underlying the pathogenesis of several reproductive diseases.

The current study was undertaken to elucidate the possible role that NF-κB may play in the pathogenesis of RP and to our knowledge the first study to date to address this topic in buffalo.

Material and methods

This study was conducted at the Animal farm, Faculty of Veterinary Medicine in Ismailia (Egypt). All animals in the farm were kept under veterinary supervision and vaccinated against some infectious diseases. The study consisted of two groups of buffalos (a, b) divided according to the time of placental expulsion: a. buffalos with normal placental release (NPR) and b. buffalos with retained placenta (BRP) for more than 12 h after parturition. According to Grunert (1983) [12] the fetal membranes were considered retained when they had not been expelled within 12h post-partum. Blood was withdrawn from the jugular vein 2-3 hours after calving. Blood samples were collected in plain clean centrifuge tubes and centrifuged for collection of sera, then stored at -20°C until assayed.

Serum samples were analyzed by the Biochemistry and Molecular Biology Unit in the Faculty of Medicine, Cairo University, for the determination of NF-κB, TNF-α, NO, PGE2, and OT levels. Commercially available Cayman CHEMICALS ELISA kits were used for the measurement of the NF-κB (catalog # 10007889), TNF-α (catalog # 589201), PGE2 (catalog # 500141) and OT (catalog # 500440). Cayman CHEMICALS colorimetric assay kit (catalog # 780001) was used for the measurement of NO.

Statistical analysis

The results are represented as mean ± SE and statistically analyzed by using one-way ANOVA. Accepted level of significance ($P \leq 0.05$).

Results

Data concerning serum levels of NF-κB, TNF-α, NO, PGE2 and OT are presented in Tables 1, 2.

BRP showed significantly reduced serum levels of NF-κB, TNF-α and NO compared to their levels in the sera of NPR animals ($P \leq 0.05$). On the other hand, the serum level of PGE2 was significantly higher in BRP than in NPR buffalos. A non-significant variation was seen in the serum level of OT between the two groups of normally released and RP buffalos.

Table 1: Serum levels of NFKβ and TNFα in buffalos with normal placental release (NPR) and buffalos with retained placenta (BRP)

	NF-κβ (µg/ml)	TNFα (µg/ml)
NPR	1.7 ± 0.098	2.47 ± 0.254
BRP	0.826 ± 0.073*	1.258 ± 0.184*

Data are presented as means ± SE of at least three replicates.
* P≤ 0.05 NPR *versus* BRP.

Table 2: Serum levels of OT, PGE2 and NO in buffalos with normal placental release (NPR) and buffalos with retained placenta (BRP)

	OT (pg/µl)	PGE2 (pg/dl)	NO (nMOL/ml)
NPR	68.95 ± 2.107	203 ± 3.175	12.2 ± 0.231
BRP	72.783 ± 10.758	231.875 ± 3.380*	9.785 ± 0.374*

Data are presented as means ± SE of at least three replicates.
* P≤ 0.05 NPR *versus* BRP.

Discussion

Results of the current study suggest that low activity of NF-κB may play a an essential role in the biochemical events associated with the pathogenesis of RP.

NF-κ B activity increases in the fetal membranes at the time of labour in human (Lim *et al* ., 2012) and in the myometrium both in human (Chapman *et al* ., 2004; Khanjani *et al* ., 2011) and rodents (Alina *et al* ., 2013). Uterine contractility seems to play an important role in the expulsion of the placenta. The studies of Janszen *et al* . (1993) [16] and Lye (1996) [26] have shown that the approach of labour in cow is characterized by increased OT and prostaglandin synthesis and release resulting in mechanical contraction of the uterus that is vital for normal delivery. Contraction persists into stage 3 of labor and is responsible for the mechanical expulsion of fetal membranes (Laven & Peters, 1996) [23].

Importantly, is the finding that activation of NF-κB increases uterine contractility and that this effect is mediated by two mechanisms. On the one hand, activated NF-κB increases expression of target genes that cause increased myometrial contractility, such as cyclooxygenase-2 (COX-2) (Terzidou *et al* ., 2011) [24] and the oxytocin receptor (OTR) (Terzidou *et al* , 2011) [24]. On the other hand, NF-κB p65 has been reported to interact with progesterone receptor (PR) to reduce its DNA binding and transcriptional activity (Kalkhoven *et al* ., 1996) [17]. In this manner, p65 may antagonize PR activation of target genes that modulate uterine quiescence. Therefore, It can be concluded that, the low level of NF-κB detected in the serum of BRP may lead to the inhibition of the functional progesterone withdrawal 'reduction of PR number'. Consequently, the expected increase in myometrial PR in BRP should of course increase the sensitivity of myometrial tissue to progesterone, thus mediating reduced myomterial contractility in these animals.

Although current data show unchanged levels of OT in BRP compared with those with NPR (table2), a role of the low level of the NF-κB in reducing OT activity is expected in BRP. Earlier studies have shown that in human parturition, in contrast to OT, the oxytocin receptor (OR) may be essential for the uterine contractile effect of OT (Blanks & Thornton, 2003) [4] and that increased levels of OT are not evident during labour (Sanu & Lamont, 2010) [32]. Actually, increased myometrial sensitivity to OT at term was reported to be mediated through increased OR expression which can be modulated by local rather than circulating, factors (Terzidou *et al* ., 2006) [24]. On the other hand, the study of Terzidou *et al* . (2006) [24] revealed that NF-κB is likely to play a role in OR expression in the amnion at term. Therefore, it is possible to suggest that NF-κB is an important factor modulating the local sensitivity to OT. The low level of NF-κB clearly suggests a mechanism by which OR may be down-regulated in the etiology of RP in buffalo.

It has been suggested that the fetal placenta must be recognized as "foreign" tissue and rejected by the immune system after parturition to cause expulsion of the placenta (Gunnink, 1984) [11]. Thus, Immune suppression is suggested to be a possible mechanism in the etiology of RP. In human, the immune response that affects placental rejection at the time of parturition involves the infiltration of leukocytes which are the source of inflammatory cytokines (Thomson *et al* ., 1999) [28]. In dairy cattle, impaired neutrophil chemotaxis and phagocytic activity were hypothesized to cause RP (Kimura *et al* ., 2003; Islam *et al* ., 2017) [15, 20]. Also, depressed production of IL-8 has been reported to be an important factor affecting neutrophil function in cows developing RP (Kimura *et al* ., 2003) [20]. Importantly, are the studies of McDonald *et al* . (1998) [27] and Page *et al* . (1999) reporting that NF-κB is activated by many of the same stimuli that elicit the production of IL-8 and other chemokines/cytokines in neutrophils. Moreover, TNF-α and other cytokines were reported to be among other factors that possess the capacity to trigger the secretion of copious amounts of IL-8 by neutrophils (Janszen *et al* ., 1993) [16]. On the other hand, the inflammatory cascade associated with parturition involves elevation of inflammatory cytokines e.g. IL-1., IL-6 and TNF-α and chemokines e.g. IL-8, CCL2 and CCL5 in amnion and myometrium (Osman *et al* ., 2003; Shynlova *et al* ., 2013) [28], [35]. Moreover, increased levels of proinflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α, in reproductive tissues, amniotic fluid (AF) and maternal serum were found to accompany both preterm and term labour (Cox *et al* ., 1997) [8]. This elevated cytokines level may account for the enhanced activity of NF-κB that accompany parturition. It is documented that NF-κB is highly inducible by pro-inflammatory stimuli including IL-1ß, IL-8 and TNF-α (Kniss *et al* ., 2001; Lappas *et al* ., 2001) [21, 22]. On the other hand, NF-κB is known to promote the formation of cytokines in many cell types (Cindrova-Davies *et al* ., 2007) [7]. Cytokine-induced NF-κB can therefore precipitate a positive feed-forward loop resulting in amplification of cytokine production and further NF-κB activation to stimulate NF-κB activity in uterine tissue. Therefore, this positive feed-forward loop may account for the reduced levels of both NF-κB and TNF-α seen in BRP (Table 1). Based on the above discussion, the reduced levels of NF-κB and TNF-α may reflect reduced neutrophil chemotaxis in BRT i.e. immune suppression in these animals, thus having a negative impact on placental release. Of importance is the finding that cytokines, especially TNF-α induce synthesis and secretion of endothelial adhesion molecules which have been demonstrated to mediate leukocyte recruitment to sites of inflammation (Sahnoun *et al* ., 1998) [31]. In turn, the induction of cell adhesion molecules (ICAM-1, VCAM-1 and E-selectin) is mediated by the activation of transcriptional factors, especially the NF-κB (Sahnoun *et al* ., 1998) [31]. On the other hand, the study of Osman *et al* . (2004) has documented an up regulation of ICAM-1 and PECAM mRNA expression in fetal membranes following labour. This points to the possible role of the inflammatory cytokines and the NF-κB in modulating the effects

of endothelial adhesion molecules in the process of parturition. Therefore, the decreased levels of NF-κB and TNF-α in BRP could be one mechanism mediating the possible down regulation of cell adhesion molecules in BRP.

Within this context, it is important to refer the results of our earlier study (Sharawi & Moustafa, 2006) [34] reporting increased levels of c ortisol in BRP. Cortisol is a powerful immune-suppressive factor that reduces the leukocyte proliferation and fundamental functions and does not allow for the normal efficient maternal immune recognition and rejection of fetal membranes (Davies *et al* ., 2004) [7].

It is worthy to mention that the immunosuppressive and anti-inflammatory effects of glucocorticoids are caused by their enhancing the production of IκB which traps activated NF-κB in inactive cytoplasmic complexes (Auphan *et al* ., 1995) [3]. Therefore, the increased levels of cortisol may account at least in part for the diminished activity of NF-κB in BRP. The increased cortisol level suggests that stress is strongly linked to the development of RP in buffalo. The increase in PGE2 in BRP (table 2) could be the source of increased cortisol level in BRP. It has been reported that PGE2 is responsible for the stimulation of the foetal hypothalamic- and hypophyseal-adrenal axis to produce cortisol and to induce parturition (Thorburn, 1992) [41]. Indeed, increased cortisol and PGE2 levels has previuosly been reported in cows affected with RP (Wischral *et al* ., 2001) [43].

It has been reported that nitric oxide plays a key role in the process leading to cervical ripening in term pregnancy in humans (Ekerhovd *et al* ., 2003) [10] and in prelabor cervical ripening in the bovine (Aalberts *et al* ., 2007) [1]. Interestingly, is the finding that the physiological expression of NOS-2 and COX-2 in trophoblasts involves a sustained activation of NF-κB which inhibition abrogates the inducibility of both genes (Callejas *et al* ., 1999) [5]. Consequently, the reduced NF-κB level in BRP may account for reduced level of NO observed in these animals. This reduced NO level may disturb the process of cervical ripening which may have a negative impact on the process of placental separation. Indeed, the study of Stjernholm-Vladic *et al* . (2004) [36] has shown that if the process of cervical ripening is disturbed, either resulting in a preterm delivery or to a prolonged delivery time.

In conclusion, the current results seem to support the claim that NF-κB may play a critical role in the pathogeneses of RP and to our knowledge this is the only study to date demonstrating this topic in buffalo.

The possible effects of the reduced levels of the NF-κB and TNF-α in reducing myometrial contractility and in causing immune suppression were discussed as potential mechanisms in the etiology of RP. Assessment of OTR and PR in the fetal and maternal parts of the placenta as well as neutrophil functions and their link to NF-κB and inflammatory cytokines in buffalos affected with RP warrant further investigation.

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