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An Overview on Placental Retention in Farm Animals

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Abstract: Fetal membranes or what is known as "placenta" is an essential organ for prenatal transfer of nutrients and oxygen from the dam to the fetus. It normally drops within short time post partum. If the placenta is not expelled within certain time (12 h post calving and 3 h post foaling), it is defined as being retained placenta (RP). Such retention creates a number of problems following pulling of microorganisms into the uterus causing its inflammation, fever, weight loss, decreased milk yield, longer calving intervals and may result in an open cow during the next year and if the infection is so bad the animal may actually die. RP causes great economic losses, mainly due to decreased milk yield and calf crop. The actual causes of RP are not clear, but the condition usually followed dystocia, maternal hypoimmunity, mal and unbalanced nutrition, stress, hereditary predispositions or infections. Since there are many causes of RP, there is no simple method for control and prevention. Basically, we have to avoid the occurrence of dystocia through genetic selection of dam and sire having minimal probability for RP, proper prepartum nutritional status and exercise. Special care should be paid for nutrition and vitamin supplementation, especially during the dry period. Maintain a sound vaccination program to minimize the chance of viral and bacterial abortions. Regular examination of the animal should be done for early diagnosis of dystocia. It could be concluded that RP is an important problem which causes great economic losses and leave the animal subfertile even after treatment and recovery. So, it is recommended to control the condition rather than to treat it.

Key words: Placenta · Farm animals · Retention · Dystocia

INTRODUCTION

Currently, the incidence of infertility becomes relatively increased with consequent reduction of productivity of farm animals. According to global Agricultural Information Network report for 2010, the total number of Egyptian cattle and buffalo decreased in 2009 and it is expected to decline in 2010 to lower level because of many problems that continue to limit the growth of the animal production industry [1]. Also, the per caput consumption of milk and its product is very low in Egypt and it should increase 300%. Egypt imports milk powder equivalent to 45000 L of liquid milk valued by $ 1.5 million. It is estimated that a yearly increase in milk production equivalent to 30 Kg per animal could replace the imported milk. Importation of dairy cattle is advocated and ministry of agriculture and other agencies have been empowered to purchase Friesian cattle, a breed which for the last few decades has proved to be able to withstand the climatic condition in Egypt [2].

Reproductive disorders, poor nutrition, infectious diseases are the main constraints of animal welfare in Egypt [3]. Retained placenta is among the main reproductive disorders in farm animals especially, dairy cattle. It causes considerable economic losses in the herd due to decreased milk production, illness and treatment cost, beside a decreased market value of the animal [4,1]. The current work aimed to throw light on the main causes of this phenomenon aiming to put guiding lines for herders to control this condition and the consequent draw backs.

Placenta: The fetal membranes or what is called "placenta" is the name given to the membranes that transfer nutrients from the dam to the fetus before birth. These membranes and blood vessels are made by the fetus and connect to the blood supply in the uterus. Across the thin connection between the membranes of the dam and the membranes of the fetus, the essential materials pass to the developing fetus. When the fetus is

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born, the placenta normally detaches within short time and is expelled. That is why it is referred to as the "afterbirth" [5]. The release of fetal membranes postpartum is a physiological process; involving loss of fetomaternal adherence, combined with contraction of uterine musculature. It is the third stage of labor and is usually accomplished within 6 hours of calving [6] and 30 to 90 minutes of foaling [7] and 49 minutes in she camel [8]. This difference in fetal membrane separation between species may be due to different forms of placentation in different species. The placenta of the mare and camel is of the diffuse epitheliochorial type with superficial villous attachment. This is a non-invasive maternal tissue at parturition. This contrasts with the more localized forms of contact in bovine and ovine placenta that is classified as chorioallantoic, villous and cotyledonary [5].

Retained Placenta: Retained placenta (RP) is the failure to expel the fetal membranes within 12 to 24 hrs after calving in bovines [9]. or within 3 hours after foaling in equines [10]. Such phenomenon is a common, albeit poorly understood postpartum disorder that has a detrimental effect on milk yield and subsequent reproductive efficiency [6].

The retention of placenta creates a number of potential problems due to the possibility of uterine infection [11]. The local infection in the uterus can cause the animal to become ill (fever, weight loss, etc). Sometimes, the infection is so bad and the cow can actually die. When the uterus becomes infected and inflamed, it takes much longer for the cow to clean and to be ready for the next breeding season. A retained placenta usually causes the cow to delay the next pregnancy for 2-6 months, late calving date in the following year and may result in an open cow next year [11]. Another result of retained placenta can be tetanus. The tetanus organism is commonly in the soil or in the feces and when it gets into the uterus; it can set up an infection and result in tetanus (lockjaw). Tetanus can be fatal and at the minimum requires 1-3 months as long term therapy [11].

Incidence: The incidence of RP varies from 2 to 10% of foaling in mares [10] and 4.0-16.1% in cow[9]. However, this incidence can be much higher in problem herds. Also, it increases during summer with increased parity, milk yield in the previous seasons and following birth of male fetus [6,12] and the breed where it may reach 54% in heavy draught than in light-weight mares [13]. Abortions, stillbirths and twin calvings resulted in increased incidence rates of 25.9, 16.4 and 43.8%, respectively. Also, El-Malky *et al*. [6] demonstrated an incidence of RP reaching 4.6% in buffalo-cows over three years of study. No available literature dealing with placental retention in camel as the reproductive performance of camels in pastoral herds was only rarely assessed and then usually based on small animal numbers [14].

Mechanism of Defective Fetal Membranes Separation: Normal expulsion of the placenta involves three components that act in concert first, placental maturation associated with the endocrine changes in late pregnancy and around parturition. Secondly, exsanguination of the fetal side of the placenta allowing shrinkage and collapse of the villi with separation from the crypts and third, uterine contractions with distortion of the placentomes [11].

The direct cause of placental retention is uncertain, but it is related to a de?ciency of myometrial contractions and failure of the maternal immune system to successfully degrade the placentomes at the end of pregnancy [15].

Established Risk Factors for Placental Retention: The key element in the pathogenesis of retained placenta is a failure of timely breakdown of the cotyledon-caruncle attachment after delivering the fetus. The most important riskfactors for placental retention are abortion, stillbirth, twining, dystocia, induction of parturition with $PGF_{2\alpha}$ and caesarean section, metabolic disorders, especially milk fever. Prenatal losses can be caused by infectious and non infectious factors. Primary attention has been often directed to infectious causes, but non infectious factors probably account for 70% or more of the cases [16]. The infectious causes of placental retention is behind the scope of this article.

Non infectious causes are often multifactorial and are difficult to diagnose [17]. The list of potential causes is quite long, however, there are a number of common causes [5].

Hereditary Causes of Retained Placenta: Recent study carried out on a herd of Friesian cows reared at lower Egypt reported that cows having blood group genotype $BGKO_xA'0'$ bred with sire have I' genotype dropped their placenta normally, while cows having $BO_3Y_2AE'_3G'P'$ genotype bred with sire having genotype I_2 showing high incidence of RP. On the other hand, the most frequent alleles in serum proteins of NRP cows were albumin (ALA), postalbumin (PalA) and amylase (AmB) gene markers, while the most frequent genetic alleles in serum proteins of RP cows were alpha globulin ($F\alpha_2A$) and

transferrin D (TFD). Moreover, it was recommended to use the above mentioned genetic constituents of both dam and sir for breeding purposes [18].

Also, mRNA and protein expressions of apoptosis-regulating factors: FAS, cellular FLICE-like inhibiting protein (cFLIP), BAX, BCL2, caspase-8 (CASP8) and CASP3 were investigated in fetal membranes either retained for more than 12 hrs or shed normally in cows. FAS mRNA expression in maternal placental tissue was less in RP cows than in non-RP cows ($P<0.05$). cFLIP mRNA expression in maternal and fetal placental tissue was greater in RP cows than in non-RP cows ($P<0.05$). CASP3 mRNA expression in maternal placental tissue was greater in Rp cows than in non-RP cows ($P<0.05$). However, the protein expressions of FAS, cFLIP and cleaved CASP3 were not significantly different between the two groups. The mRNA and protein expressions of BAX, BCL2 and CASP8 were also not significantly different between the two groups. In the immunohistochemical study, single-stranded DNA which appears specifically in the apoptotic cells was mainly found in the maternal placenta of non-RFM cows. Together with these results, It was suggested that RP occurs at least in part due to a dysfunctional apoptotic process caused by the inhibition of FAS expression in the maternal placenta and the increase of cFLIP expression in the maternal and fetal placenta [19].

On the other side, Cyp19 gene encodes the aromatase enzyme responsible for catalyzing the rate limiting step in biosynthesis of estrogen, an important hormone for placental maturation and expulsion has been recently studied. Comparative analysis of Cyp19 gene expression and its epigenetic regulation in placental cotyledons of animals with and without RP revealed significantly lower expression of Cyp19 gene in placental samples of RFM affected animals in comparison to normal animals. Methylation analysis of 5 CpG dinucleotides of placenta specific Cyp19 gene promoter I.1 and proximal promoter, PII showed hypo-methylation of both PI.1 and PII in term placenta of normal and diseased animals. In conclusion, a mechanism other than promoter methylation is responsible for decreased aromatase expression in placental cotyledons of animals suffering from RP [20].

Defective Hormonal Function: Placental separation occurs when foetal cortisol induces the production of the enzymes, 17_{α}-hydroxylase and aromatase in the placenta which favour oestrogen synthesis at the expense of progesterone synthesis. Maternal plasma levels of oestradiol-17_{β} increase suddenly, while plasma levels of progesterone decline sharply immediately prior to parturition. It is supposed during the week before parturition, the level of estradiol reaches its maximum level to help the uterus to get rid of any remnant of fetal membranes. Therefore, a decreased level of estrogen may be indicated as a factor enhancing RP [21].

Spontaneous myometrial contractility is augmented by autocrine and paracrine release of $PGF_{2\alpha}$ and parturition ensues. Disturbed endocrine function, high progesterone and cortisol levels and low oestradiol level was traced in the blood cows with RP [22]. Increased progesterone level in RP may be due to failure of the placenta to produce specific steroidal enzymes that help in progesterone aromatization and its conversion to oestrogen [5].

Failure of Maternal Immune Response: Maternal immunological recognition of fetal MHC class I proteins expressed by trophoblast cells triggers an immune/inflammatory response that contributes to placental separation [23]. This lymphocytic activation was suppressed at the foetomaternal interface alongside the pregnancy course to avoid rejection of fetal allograft [24] where the trophoblast secretes interferon-tau (IFN-τ) and both trophoblast and endometrium secrete prostaglandin E_2 and the endometrial glands secrete serpins (uterine milk proteins), all of which inhibit lymphocyte activation to keep on the embryo not rejected by the dam. Furthermore, for successful non-classical pregnancy, class I MHC antigens expressed by trophoblasts prevent maternal NK-mediated cytotoxic responses [25]. Strong impairment in polymorphonuclear neutrophils (PMNs) chemotaxis was demonstrated in placentomes from cows with retained placentas. Blood leukocytes and neutrophils of cows with retained placenta were less reactive to chemotactic stimuli than in cows with normal placental separation [26]. Acyloxyacyl hydrolase (AOAH) is an enzyme of PMNs capable of detoxifying endotoxin of the coliform bacteria causing severe mastitis and metritis [27]. It is found that AOAH in RP cows is less than non-RP cows. Decreased myeloperoxidase activity was reported in circulating neutrophils of cows with RP [28]. Furthermore, higher blood lysozyme and acid phosphatase activities were reported for cows with acute in?ammatory responses and NRP [29]. The increased cortisol concentrations in cows that developed RP Ahmed *et al.* [12] has immunosuppressive and inhibitory effects on leukocyte migratory activity [30]. Preparturient endocrine changes are supposed to interfere with the innate immunity of the dam [31]. High progesterone and cortisol levels in the

blood in stressed cows [22] may induce the accumulation of immunosuppressive proteins in the uterine lumen which make the uterus susceptible to infection and persistence of bacteria [32].

In high lactating cows, somatotropins stimulate the production of insulin-like growth factor 1 (IGF-1) in the hepatic cells. However, the plasma IGF-1 level was found to be quite low, especially in the hyperketonaemic animals [33] in which metabolic products (non esterified fatty acids (NEFA) and βOH-butyrate (BHB) accumulate and impairs the migration, phagocytic and killing activity and /or the oxidative burst of PMNs and other leukocytes, enhancing the susceptibility of host to invading pathogens [34].

Mechanical Causes of Retained Placenta: Difficult birth (calf too large for cow, backwards calf known as breech birth, one leg or head backwards), twins, late or premature birth, prenatal loss, induction of parturition with $PGF_2\alpha$, cesarean section and fetal monsters or emphysematous fetus (gas-filled fetus) are direct causes of dystocia and consequently to RP.

Nutritional Causes of Retained Placenta: Cows 0-120 days postpartum are at risk of ration formulation error and feed delivery problems. Periparturient dairy cows (0-20 days postpartum) undergoes a transition from a relatively high ?ber diet to a lactation diet that generally is higher in energy and lower in ?ber. During this period, the amount of energy required for maintenance of body tissues and milk production usually exceeds the amount of energy in the diet [35]. This nutrient de?cit makes the cow susceptible to metabolic diseases such as ketosis and milk fever, which usually occur within 3 weeks of thereby calving slowing adaptation to the postpartum diet. Also, prepartum heavy grain feeding may be associated with both higher milk production and a higher incidence of left-displaced abomasum [36] and increased risk of reproductive disorders such as dystocia, retained placenta, cystic ovaries, metritis [37] prolapsed uterus [38] and acute clinical mastitis increasing the risk of non-parturient paresis [39]. Cows being adapted to high-energy and high concentrate lactation rations are at risk because the rumen papillae need time to elongate and the microbial population needs time for adaptation. Also, lower dry matter intake near calving can result in depressed forage intake.

It has generally been found that other feeding-related diseases increase the risk of non-feeding-related diseases rather than vice versa.

Vitamin and mineral deficiency conditions such as selenium, vitamin E and vitamin A, β-carotene and disturbed C/P(1.5/1) ratio can impair general immunity [40] and may alter the competence of cellular self-defense mechanism and can increase the risk for placental retention and metritis.

Ahmed *et al.* [41] reported that RP was associated with oxidative stress as shown by the obvious increase of blood malondialdehyde and nitric oxide and decreases of catalase, superoxide dismutase, ascorbic acid, glutathione reduced and total antioxidant capacity values with low zinc, copper, iron and selenium concentrations. It is concluded that RP is associated with stressful condition in buffaloes and the condition lead to high incidence of infertility and culling, especially when it was associated with uterine fibrosis.

High milking cows with a greater degree of negative energy balance prepartum and higher NEFA concentrations were 80% more likely to suffer from RP [42]. On the other side, over-conditioned cows were shown to be more sensitive to retained placenta and subsequent infertility than cows with normal body condition scores [43] etiological mechanism for retained placenta was associated with dietary-conditioned liver disorders and high plasma urea and gamma-glutamyl transferase concentrations [44] Also low plasma glucose and PGFM (the main PGF2alpha metabolite) levels, Low monocyte and high red cell counts was traced in blood of cows with retained placenta, especially in the late gestation period [45] and could be connected with the disease by means of dietary unbalanced ratio of n-3/n-6 polyunsaturated fatty acid (PUFAs) that would involve reductions in synthesis of cyclooxygenase products, impair uterine contractions, vascular tone and platelet aggregation leading to retained placenta [46].

Managemental Causes of Retained Placenta: Myometrium contractility is the third component of self defence mechanisms, since uterus contractions expel the uterine content. Lack of exercise and hypocalcemia are the most frequent causes of decreased myometrium contractility [40]. However, in a study by [47] there was no correlation between blood ionized calcium (ca+2) concentrations and any of the contractility parameters. Also, the lack of uterine motility plays little or no role in the occurrence of retained placenta. Moreover, cows with retained placenta have normal or increased uterine activity in the days after calving [15].

Stress (Transportation, rough handling, poor feed conditions, Isolation from group, Lameness,) results in elevated corticosteroids and increased risk of placental retention. Obesity may lead to dystocia and subsequent RP.

Economic and Reproductive Consequences of Retained Placenta: In dairy cows, retained placenta may be the cause of serious economic losses to the farmers as cows with retained placenta may develop bacterial infection and become ill and thus reduce production. Some may even die. Milk from cows with retained placenta is unfit for human consumption and therefore cannot be sold. The fertility of dairy cows is affected when most cows in the herd suffer from retained placenta. This causes a direct loss to the farmer due to delayed calving leading to a lengthy period between births (calving intervals) and hence low milk production. It is unhygienic to milk a cow with a decomposing afterbirth hanging on it [42]. In mare the very rapid passage of the placenta, i.e. within 10-15 min, may also be considered abnormal. Many of these placentae have signs of substantial placentitis or premature placental separation. Adverse events that center around foaling and uterine involution are likely to contribute to acute and chronic endometritis, susceptibility to uterine infection and persistent post mating endometritis [5]. During an uncomplicated foaling but more frequently during an aggressively assisted foaling or dystocia, the cervix may be damaged or lacerated. Cervical lacerations can range from minor to full thickness. Furthermore, the vestibulovaginal sphincter may be damaged during foaling, particularly dystocia. The integrity of the vulva lips, vestibulovaginal sphincter and cervix are substantial barriers to uterine infection and contamination. When these structures are damaged, there is both increased microbial challenge to the reproductive tract and increased potential for altered clearance of ?uids and bacteria following foaling or mating [10].

The financial losses due to retained placenta in dairy cattle were estimated by using a data-set containing the birth records provided data on the reproductive performance of cows with and without retained placenta. The fertility of cows after retention of the placenta appeared to be affected.

An economic calculation made by adding the losses due to increased calving interval, increased culling rate, loss of milk production and the costs of veterinary treatment and drugs revealed that the total loss due to retained placenta for a 100-cow farm with an average incidence of the condition (6.6 per cent) was 471 Dutch pounds per year. For a 'problem' farm with a 30 per cent rate, the loss was 2139 Dutch pounds per year [49]. Sensitivity analysis showed that the greatest financial losses were caused by loss of milk production, followed by the number of animals suffering from complications. In a study, a?ected animals produced 355 L less milk than normal cows during the ?rst 60 days of lactation[50]. While in buffalo cows it was 776 L lower than normal ones during milking season [6]. The reduction in milk yield persists even after resolution of the problem. Buffalo groups with Rp reduced its milk yield by 15.79% than the preceding season while non retained placenta (NRP) group increased milk productivity by 6.99% over that of the preceding season [51]. Buffalo-cows showed longer calving interval in days (411 and 541), more days open (101 and 231) and increased number of services per conception (1.3 and 3.5) in NRP and Rp dams, respectively [6]. Cows with the consequent clinical endometritis between 20 and 33 days after calving took 27% longer to become pregnant and were 1.7 times more likely to be culled for reproductive failure than cows without endometritis[34].

Optimal Strategies for the Management of Herds with RP: As the non infectious causes of placental retention is multi factorial and difficult to be diagnosed, especial care should be paid for control measures rather than treatment protocols. The genetic aspect should be put in consideration to select animals having the minimal probability for the occurrence of RP [18]. One of the useful tools for selection is the birth weight. Lighter birth weights will decrease calving problems if all other factors are equal. However, it is also important to remember that big cows with big pelvic canals can have big calves easily. So selection of bulls with suitable birth weight for the breed is essential to protect the herd from calving problem [53]. Use of modern techniques, such as, superovulation, collection of ova, *in vitro* fertilization and cryopreservation or embryo freezing, for super genetic animals having minimal incidence of reproductive disorders may help to overcome such problems. Some progress has been made in the field of embryo freezing and transfer in dromedary camels [54]. and recently, in embryo transfer and oocyte recovery in llamas [55, 56] and alpacas [57]. There have been some attempts to hybridize old and new world camelids using assisted reproductive techniques [58]. *In vitro* maturation and fertilization have been extensively studied in many domestic species such

as cattle [59], buffalo [60], sheep [61], goat [62], pig [63] and horse [64]. Only few studies in this respect are available in Camelus dromedaries [65].

Nutrition: Planning is the ?rst step in the herd health cycle and should focus on appropriate objectives. Advisors such as nutritionalists are needed beside the veterinary services[66]. Body condition scoring (BCS) is a simple method for evaluating the adequacy of the dietary energy inputs, Both high and low BCS before calving can be deleterious [67]. Supplementation with balanced vitamin and mineral mixture in prepartum period is considered a prophylactic step to avoid fetal membrane retention. Elevated BHBA and subclinical ketosis in dairy cattle should be controlled before calving. Injection of Catosal® (10% butaphosphan and cyanocobalamin) on the day of calving and 1 day later may decrease the prevalence of subclinical ketosis during the week after calving in mature dairy cows, but not in first-and second-lactation animals [68]. Prepartum supplementation with antioxidants, vitamin E (DL α-tocopherol acetate, 1100 IU) and Se (sodium selenite, 30 mg) by single im injection, at 3 week prepartum, is used as a prophylactic dose to avoid placental retention in cows[68] and buffaloes[69]. Intramuscular injection of vitamins AD_3E once a week during the last month of pregnancy induced 100% placental dropping in local Egyptian Friesian cows with favorable time for completion of uterine involution, onset of first post partum estrus. And conception [69].

Use of Ecbolics and Immunomodulators: Use of ecbolic, like oxytocin, helps in inducing myometrial contraction. it was evident that *E Coli* LPS plus oxytocin effectively reduced the uterine in?ammation and infection, thus increasing the overall reproductive efficiency in chronically sub fertile mares. LPS is a potent secretagogue for a variety of in?ammatory mediators and immune regulatory cytokines from endometrial cells and leukocytes [70]. The failure to produce any systemic endotoxic responses after intrauterine infusion of E coli endotoxin confirms that *E coli* LPS can be used safely without producing any adverse reaction in mares [71]. Recombinant human interleukin 8 (rhIL-8) was effective in attracting PMNs into the uterus within 6 hours after administration in cattle and horses [72].

Ethno Veterinary Practices: Ethno veterinary practices (EVPs) still have signi?cant contributions to animal health and are regarded as sustainable veterinary medicine in the new era [73]. The ultimate consideration for EVPs is due to its greater accessibility, lower cost and apparent effectiveness [74] (Mwale *et al*., 2005). In case of retention of fetal membranes, it was a general perception among the traditional veterinary healers (TVHs) that it is a result of constipation, weakness and dif?cult birth (dystocia). Therefore, jaggery, milk and oil were often administered as energizers. Camel's milk was given preference over milk from other species owing to its supposedly high mineral content derived from weeds and shrubs. Different species of plants were used with the perception that these are heat generating (calori?c) and purgative. Common salt was rubbed on the back of animals for expulsion of fetal membranes with the perception that it has a stimulatory effect on the uterus [75]. *Sesamum indicum, Trachyspermum ammi*, *Linum usitatissimum, Dalbergia sissoo, Rosa indica, Brassica campestris and Brassica rapa* are used for genital prolapse, retention of fetal membranes and dystocia associated with incomplete cervical dilatation [75].

It is concluded that retention of placenta is an important reproductive disorder in farm animals, especially in dairy enterprises. The actual causes for RP is still not clear, however, many factors can predispose for the condition, especially those are associated with uterine inertia, dystocia and abortion. The condition causes great economic losses in the form of low milk yields, uterine infection, long calving interval, with subsequent subfertility even after treatment and recovery, beside the cost of drug and veterinary intervention. On the other hand, due to the multifactorial causes, it is recommended to control the condition rather than to treat it. Special care should be paid for nutritional and managemental programs.

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