

## UROVAGINA: A CAUSE OF ENDOMETRITIS AND INFERTILITY IN DAIRY COWS: A REVIEW

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**Abstract:** *Infertility is one of the common problems in dairy herd, which prevents the one calf crop per year. Urovagina play a significant role in creating infertility in mare as well as in cow by causing necrotizing endometritis and making the uterus unsuitable for spermatozoa and embryo. Diagnosis and treatment options to correct the urovagina and its reproductive outcome in mare are well established. The information regarding urovagina and evidence of economically more pragmatic treatment is lacking in cows. Therefore, the aim of the current review was to review the effect of urovagina on fertility of dairy cows and treatment modality especially more pragmatic and economically viable under field condition.*

**Key words:** Urovagina, Endometritis, Cow

### INTRODUCTION

Recent statistics on infertility among dairy cows is increasing in trends and cause huge economic losses to the dairy farmers in India as well as in other countries [1,2]. Etiological responsible for causing infertility is amalgamated in nature. Urovagina is one of the important conditions, causing serious cause of infertility in dairy cows [3,4] next to mare [5]. It is also known as vesico-vaginal reflex or urine pooling, refers to the presence of urine in the cranial vagina and possibly in the uterus, gives rise to vaginitis, cervicitis and endometritis, ultimately result in infertility [3,4,6]. An increasing number of cows are being diagnosed with urovagina, and there seems to be a greater prevalence in certain breeds, predominantly Charolais and Holstein [6]. In order to palliate the urovagina associated infertility, knowing

about its etio-pathogenesis, diagnosis and its correction procedure by using both conservative and surgical procedure.

### CLASSIFICATION OF UROVAGINA:

The vaginal evacuate have urine or urine-mixed mucus (characterized by at least any two of the following: yellowish in color, watery in consistency, uremic in smell, and pH>7.4) were defined as urovagina. The cases of urovagina are classified into mild, moderate, severe degree according to the extent of covering of the external cervical or by the urine or urine-mixed mucus [7].

In mild degree of urovagina, small amount of urine or urine-mixed mucus only on the floor of the vagina but not covering the external cervical os whereas in

moderate degree of urovagina, appreciable amount of urine or urine-mixed mucus covering up to half of the external cervical os (some risk of urine entering into the uterus). But in severe degree of urovagina, large amount of urine or urine-mixed mucus are covering at least half (up to the entire) external cervical os (high risk of urine entering into the uterus).

Moderate and severe degrees of urovagina are appreciably related with reduced pregnancy rate but the mild degree of urovagina is not associated with decreased pregnancy rate. Furthermore, there is no significant difference in pregnancy rates between cows with moderate and those with severe degrees of urovagina. Therefore, moderate and severe degrees of urovagina are combined and defined as clinically relevant urovagina. The prevalence of clinically relevant urovagina among herds ranged from 4.8% to 20.6% [8].

### **RISK FACTORS FOR UROVAGINA**

**Parity:** This condition occurs most generally in old pluriparous cows and mares with poor vaginal and vestibular structure resulting from multiple pregnancies. After repeated calvings, aged animals have increased abdominal girth and weakened abdominal musculature, resulting in cranial displacement of the genitalia due to tension [3,9]. Urovagina has higher prevalence in first-calf heifers than in other parities. The first-calf heifers are at six folds higher risk than the other parities. This is for the reason that first-calf heifers have privileged risks of dystocia [10]. Trauma and contusion of the vaginal canal occur during dystocia. Others also have reported that dystocia is a risk factor for urovagina [3,9,11].

**Cystic ovarian degeneration:** The vesicovaginal reflex condition is mostly restricted in Charolais and Holstein [6]. In cow, affected with cystic ovarian diseases causes relaxation and cranioventral tipping of entire reproductive tract and end-up in urovagina [12].

**Dystocia:** Dystocia is the risk factor for urovagina in dairy cows. Severe dystocia damages the constrictor vestibule muscle, vulval suspensory ligament and anal retractor muscle leading to urine backflow and pooling at the cranial vagina [3,6]. The weight of urine in this area may then cause cranioventral displacement of the vagina toward the

brim of the pelvis, further complicating the problem [3,13].

**Cows with endometritis:** Cows with endometritis within 60 d postpartum are more likely to be affected with urovagina than are those without endometritis. Endometritis might have increased uterine weight (due to edema, fluid accumulation, or delayed involution), which then might have caused cranioventral displacement of the vagina, leading to urine flowing cranially and thus causing urovagina [8].

**Body condition score:** Thin cows (BCS<2.5) are more likely to be affected with urovagina than normal cows. Thin cows have loose vaginal walls due to loss of perivaginal fat, which may result in ventral displacement of the vagina and the external urethral opening to be positioned at a level higher than the floor of cranial vagina, leading to urine pooling in the vaginal fornix [8].

**Sex of the fetus:** Male-calf deliveries also are at higher risk as dystocia. The association between delivery of a male calf and injury is higher in older cows than in first-calf heifers. Male calf deliveries produces higher risk of rectovaginal injuries, because males typically are heavier than the female calves, they cause more dystocia and damage to the birth canal [10].

**Orientation of vulva and slopping of pelvic girdle:** Horizontal vulva is significant risk factors for a cow to be diagnosed with urovagina [8]. Cranioventral tipping of the pelvis causes the external urethral orifice during urination to be higher than the adjacent vaginal floor. This results in urine gravitating into the vaginal fornix [11,14].

### **ETIO-PATHOGENESIS**

The precise causes of the condition are not known. However, the condition is instigated by either congenital or acquired factors [11]. The acquired factors like dystocia [3], rectovaginal injuries [10], endometritis [8] and cystic ovarian degeneration [12] are bringing about anatomical changes in the tubular genitalia of the cow, such as the cranioventral displacement of the vagina, cervix and uterus. The vulva is displaced above the level of the pelvic brim in a nearly horizontal position, so that the external urethral orifice during urination to be higher than the

anterior pelvic floor. This results in urine gravitating into the vaginal fornix and lead to urine pooling, a condition known as urovagina. The agglomerated urine and vaginal debris provoke vaginitis and cervicitis [3,11,14].

The cervix shows patency during estrum and immediately after postpartum. During this period the volume of urine is transcended to the level of cervical lumen lead to urine passage into the cervix and uterus that ultimately upshot in superficial endometritis [15] and necrotizing endometritis [4], and thus might have caused infertility due to spermicidal action of urine [5,11] and early embryonic mortality due to adverse uterine environment. When vesicovaginal reflux is allowed to persist (moderate and severe degrees), the resultant persistent or chronic endometritis may lead to periglandular fibrosis as well as premature regression of the corpus luteum, causing a deficiency of progesterone and early embryonic loss [4,16]. Urovagina has significant effects on the prevalence of endometritis after 60 d postpartum [8]. The prevalence of endometritis during the later postpartum period is significantly higher in cows with severe and moderate degrees of urovagina but not in cows with mild urovagina [8].

Based on their impact on reproductive performance, moderate and severe degrees of urovagina are defined as clinically relevant urovagina, which is associated with 53% reduction in pregnancy rate per insemination. Consequently, cows with clinically relevant urovagina require more service per conception and thus have more days open. In this connection, cows with clinically relevant urovagina are about 7 times more likely not to get pregnant within 210 d, five times more likely to be culled for any reasons, and 10 times more likely to be culled for reproductive reasons than are cows without urovagina. In contrast, mild urovagina do not impair any of the reproductive performance end points [8].

## CLINICAL SIGNS

Horizontal vulva is a characteristics sign to identify the urovagina condition. The vulva is displaced above the level of the pelvic brim in a nearly horizontal position with the critical angle by 45° and more [7]. Other obvious accompanying signs are perineal atrophy due to perianal fat and connective tissue atrophy, cranial sinking of the anus and cranio-ventral tipping of pelvic girdle and insufficient closure with

inverted vulvar lips [8]. Dribbling of urine from the vulva, which soils the perineal area and it predisposes to attack of bacteria and maggots. Reduced conception rate, more services required for per conception, more service periods are the important indicators to rule out the urovagina associated infertility problems. Chronic persistent or intermittent muco-purulent discharge is observed in case of urovagina-induced endometritis [4,8].

## DIAGNOSIS

**Vaginoscopy:** First the cows are restrained in a feeding stanchion, the tail is held to one side, the vulva is washed with lukewarm water mixed with mild antiseptic, wiped with a clean paper towel, sprayed with 2% PVP-iodine, and wiped with cotton soaked in 70% alcohol. The autoclave-sterilized glass vaginoscope is inserted into the vagina upto the level of the external cervical os. The contents of the vagina or the external cervical os are viewed using a torch light and a portion is aspirated with a 25 ml or 50-ml syringe fitted to an AI gun sheath and transferred into a petri dish. The recovered fluid is checked for its color, consistency (watery or mucoid), smell (uremic or no smell), and pH. During vaginoscopy, congestion (severe, mild, or none), protrusion (severe, mild, or none), and degree of opening (open or closed) of the external cervical os are also recorded. After each visit, all vaginoscopes used are washed, wrapped individually and autoclaved [7].

**Rectal examination:** Manual pressure is applied dorsally from the rectum to the vagina from anterior to posterior and the animal is watched if there is urinary leakage from the vulva. The signs of endometritis such as thickening of the cervix, positional alterations of the uterus, symmetry of the uterine horns, mural thickening, luminal content (presence of fluctuation and fluid), and alterations of the oviducts, sizes and functional bodies of the ovaries are also determined [7,8].

**Ultrasonographical examination:** Ultrasonography is used to detect the urine accumulation in the vagina as well as fluid or pus in the uterus. Endometritis or metritis is diagnosed in cows with pathological fluid in the uterus. Diameters of the cervix and uterus are recorded. Changes of the echogenicity such as reduced echogenicity due to uterine edema or increased endometrial echogenicity are evaluated. A diameter larger than 5 cm of the cervix or of at least

one uterine horn or a difference in diameter of more than 1.5 cm between both uterine horns is interpreted as suspicious for cervicitis and/or endometritis. COD is one of the pathological condition which predisposing for urovagina can be effectively diagnosed based on the size of follicle or CL exceed 2.5cm, categorized as COD [6,7].

**Other physical parameters:** The angle of pelvic girdle and the orientation of vulva are determined. The angle of pelvic girdle is determined by putting a long rigid scale over the tuber coxae and tuber ischii and classified as horizontal (tuber coxae and tuber ischii at the same horizontal level), caudoventral (tuber coxae at higher, level than tuber ischii), or cranioventral (tuber coxae at lower level than tuber ischii). The orientation of the vulva is determined by visual inspection of vulval lips. A cow with vulval lips angled  $>45^\circ$  with the vertical line is classified as having a horizontal vulva, whereas the remaining cows are classified as a vertical vulva [7].

## TREATMENT

Treatment of urovagina may be conservative or surgical. Decisions about conservative versus surgical treatment are made based on the value of cows, severity of the problem, success of previous treatments, the stage of lactation, and the number of failed inseminations.

Conservative treatment of urovagina consists of evacuation of pooled urine by ventral and caudal pressure through the rectum before insemination. Insemination is performed by a double sheathing technique. Some practitioners also recommended another evacuation 24 hrs post breeding followed by intrauterine antibiotic infusions. Many cows with mild to moderate urovagina can be bred successfully in this manner, although two or three repeat services are not unusual. Recently, the ozone treatment is found to be the most effective treatment modality, resulting in the shortest period of days open (79 days), the fewest number of inseminations until pregnancy (1.63) and the smallest number of culled cows [17]. The ozone flush coupled with intracornual insemination presents an effective treatment option for urovagina that can lead to successful conceptions and pregnancies in dairy cows [17].

Invasive surgical correction of urovagina may be necessary when the cow is extremely valuable or

when conservative efforts have failed to allow conception. Various surgical techniques have been described to correct urovagina in mares, including the transverse fold technique, the McKinnon technique [4] and the Brown technique [5,14,18]. These same techniques have been modified to correct urovagina in cows [4,11,19]. Modification of these techniques for cows is necessary because cows, unlike mares, do not have a prominent urethral fold that can be incorporated into the extension. The most common techniques used to treat affected cows are the transverse fold technique [11,19], a modified McKinnon technique [4,19], and a modified Brown technique [3]. The incidence of failure using the transverse fold technique has not been reported, but the technique is laborious and may interfere with coitus. The incidence of failure using a modified McKinnon or a modified Brown technique to prevent urovagina is high, because a fistula often forms in the mucosal extension of the urethra [3,19,20] and temporary paresis of the bladder [4]. When creating a urethral extension, a modified McKinnon technique may be superior to a modified Brown technique because a modified McKinnon technique provides a more spacious extension and is less likely to decrease the circumference of the vestibule [19,21]. The cow conceived promptly after insemination, but suffered similar injuries subsequently during calving [4].

**Transverse fold technique:** Transverse fold technique was reported by various authors [11, 19]. A transverse dam composed of a fold of vaginal mucosa is established cranial to the external urinary meatus in order to prevent cranial flow of urine. Surgery is done under low epidural anesthesia with the cow in the standing position. Urine and debris are removed from the vagina by hand. The perineal region and vaginal vault are completely and repeatedly flushed with povidone iodine surgical detergent. In order to deflate the greatly enlarged vagina, the cranial limit of the vagina is depressed, grasped with one hand and retracted, thus forcing out the air. Deflation is transitory, but it is helpful in elevating a transverse fold of mucosa on the vaginal floor. The fold should include the ventral  $120^\circ$  of the circumference of the vagina and is fixed in position by using a 12cm half- curved, cutting edge needle and no. 2 non absorbable suture material in a continuous horizontal mattress pattern through the base of the fold. Approximately 8cm of vaginal floor should be elevated to form the fold. As the pattern is carried across the midline, urethra must be avoided.

The continuous suture is drawn taut, thus forming semi lunar dam about 5cm high. The intent is to cause adhesion of the base of the fold for permanent structural change. Fortunately, the free edge of the dam tends to lean cranially, a safety factor for both cow and bull during coitus, should natural services be used. The suture is removed in 30 days. The vagina is infused daily with a bland, oil-based antibiotic ointment.

If concomitant perineal injury causes pneumovagina, repair is required using the applicable techniques previously described. In the absence of primary pneumovagina, when only the dam is employed, the pooling of urine should cease immediately. This may be checked by manual rectal examination. The vagina, relieved of the continuing insult, should return to normal condition and tone.

**Modified McKinnon technique of urethral extension:** Modified McKinnon technique of urethral extension was reported by Fubini and Ducharme [19], Gilbert et al. [4]. Surgery is performed under sedation and epidural anaesthesia. The tail is bandaged with rolled gauze and secured dorsally to the chute, and the perineum is prepared for surgery. Before performing a modified McKinnon technique of urethral extension, the midpoint between the dorsal and ventral commissures of the vulva is marked on each labium by creating a small nick, with dissecting scissors, at the mucocutaneous junction. The lumen of the vestibule is exposed using a vaginally placed Finochietto retractor with custom-made blades, 22-cm long and 4-cm wide. The retractor is positioned so that the bottom edge of the blades rested at the labial nicks created midway between the dorsal and ventral commissures of the vulva and opened to expose the lumen of the vestibule and vagina. A Foley catheter is inserted into the bladder through the external urethral orifice, and the cuff inflated, to ensure that the surgical field remained free of urine during the procedure. Using a #15 scalpel blade on a 24-cm long, 90°- angled scalpel handle, the mucosa 2 cm cranial to the caudal border of the urethral opening is incised transversely. The incision is continued laterally and dorsally to the bottom edge of the blades of the retractor and then continued caudally along the bottom edge of each blade on each side of the vestibule to the small nick created on each labium. The incision is then directed ventrally along the mucocutaneous junction of each labium, to a point halfway between the nick in the labium and the ventral commissures of the vulva. The submucosa

at the ventral margin of the incision is undermined on both the right and left aspects of the vestibule with scissors to form a U-shaped flap. Dissection is continued ventrally until the right and left sides of the edge of the flap could be reflected to the midline without tension. The right and left sides of the edge of the mucosal flap are apposed with 2-0 polydioxanone suture placed in a continuous Lembert pattern to create a mucosa-lined tunnel that extended from 2 cm cranial to the external urethral orifice to the mucocutaneous junction of the labia. A second, re-enforcing suture line, using the same suture and suture pattern, is placed over the first suture line, and the Foley catheter is removed. Submucosa exposed on the ventral aspect of the vestibule by creating the vestibular flaps is either allowed to heal by secondary intention is covered with a free, unmeshed, mucosal graft, obtained from the dorsal aspect of the vestibule. Application of a mucosal graft to the subcutaneous tissue exposed to the vestibule using the McKinnon technique of creating a urethral extension in cows is of little or no benefit in preventing the formation of a fistula in the extension [21].

**Modified Brown technique of urethral extension:** Modified Brown technique of urethral extension was reported by various authors [3,11] and this technique is derived from a surgical technique developed by Brown and colleagues for mares. The patient may be sedated, and epidural anaesthesia is administered. Single or double layer mucosal canal is structured following a vaginal mucosal incision starting at the transverse fold at the cranial urethral opening into the vagina. The incision is then continued as a U-shaped mucosal incision to a point 2.0 to 2.5 cm from the vulvar lips. The mucosa then is undermined both dorsally and ventrally from the incision line to loosen and relax tension on the mucosa, which is then apposed over a Foley catheter placed in the bladder and exiting the urethra. A continuous Lembert suture is used for closure of the ventral and then dorsal layers. The surgery can be completed successfully in a single layer, using only the ventral tissue flap. The exposed submucosa usually granulates and re-epithelialises without any complication.

Closure in a single layer has the advantage of preserving a larger vestibular lumen, with less likelihood of re-injury at subsequent calving. Systemic antibiotics and NSAIDs are administered preoperatively and post operatively. It is preferable

to leave a Foley catheter in place for 48 to 72 hours after surgery because some cattle initially fail to urinate spontaneously following the procedure. It is wise to continue the systemic antibiotics and place a Heimlich valve on the exposed end of the Foley catheter to minimize the chance of ascending urinary tract infections. An alternative surgical technique involves a buried Purse – string suture at the level of the vestibule-vaginal junction, Taking care not to penetrate the urethra, which should be catheterized so that its location is clear. The suture tightened to allow a lumen of only 2 to 3 cm. The surgery is simple, is rapid, and has resulted in a high success rate in a small number of cows to date.

#### **Vestibulovaginal cerclage technique:**

Vestibulovaginal cerclage technique is a new surgical technique and by using this surgical technique for urovagina correction could dramatically increase the fertility of cows [13]. Surgery is performed on standing animals under epidural anesthesia. Once the animal is anesthetized, the tail is tied to one side by a neck rope. Pneumovagina is induced by separating the vulvar labia, and feces are removed from the rectum. The rectum is then packed with cotton and both the perineum and vaginal vault are gently cleaned with a mild, organic iodine surgical scrub. Absorbable No. 2 polydioxanone suture threaded through a 10-cm curved, cutting-edge needle is used to create the cerclage. The lumen of the vestibule and vagina are exposed by keeping the vulvar labia open using forceps. An incision 5–10 mm long using Metzenbaum scissors is made at a 4 o'clock position in the caudal portion of the vestibulovaginal junction. This incision is then extended to a depth of about 1 cm by blunt dissection. The needle is introduced through the incision and passed cranially to the urethral opening between the vaginal wall and the urethra along the vestibulovaginal junction floor until the 8 o'clock position of the ring, where the needle is exposed and withdrawn. Care is taken to avoid incorporating the urethra in the suture line, by placing one finger in the urethra during this maneuver. The needle is then reentered at the 8 o'clock position and passed under the vaginal wall to the 12 o'clock position, and finally the suture is passed from the 12 until the 4 o'clock position of the vestibulovaginal junction. It is ensured that the two ends of the suture exited the incision pass at the 4 o'clock position. Tension is applied to each end of the suture to create the vestibulovaginal cerclage. The suture is tightened to permit the entry of one finger in the vagina and a surgeon's knot is

used to maintain closure. The knot is embedded at the incision point so that the suture line is completely under the mucosa. When the modified needle is used, the needle is withdrawn and threaded at each step.

The cows received no antimicrobial treatment. A gentle finger examination of the surgical site is performed from the vestibular side on the day after surgery to detect possible suture dehiscence or complete failure of the suture closure, and to assess the function of the constrictor vestibule muscle by stimulating the urethral orifice. The same examination is performed 20 days after surgery to assess healing. In cows that became pregnant, a further examination is performed in late gestation and parturition is closely monitored. One of the primary disadvantages of the vestibulovaginal cerclage procedure is that natural service is not possible for at least 2 or 3 months, which is the time, required for suture resorption. By using this surgical technique for urovagina correction could dramatically increase the fertility of cows.

#### **PREVENTION**

Dystocia is the major risk factor, which instigates the urovagina. Handling of dystocia with care might have a generous role in reducing rectovaginal injuries particularly urovagina. First-calf heifers are more prone for dystocia. In order to assuage this condition, the first-calf heifers to be monitor under close observation and care during calving mandatory. Urovagina reoccur in the next calving, cows with the history of these this condition should be monitored at the next labor. Reducing the dystocia has a substantial role in plummet the occurrence of these injuries and it can be obtained by inseminating heifers with best age, weight and height by easy-calving sperm [9]. Efficient pre-calving diet to prevent over weighting heifers and cows, and refuse excessive and impatience extraction during the second stage of labor, allowing ample time for dilation of the soft tissues of birth canal once delivery is initiated, should not be overlooked. Thus, we recommend the usage of bull semen of bulls rated for calving ease after the surgical repair. During an obstetrical procedure, while the vulva in dilation interferes appreciably with delivery of the fetus, episiotomy is indicated to avoid serious and costly injury to the perineum. Cows with the history of cystic ovarian degeneration, endometritis and pneumovagina should be cured by prompt diagnosis. Hereditary predisposition of urovagina among Charolais and Holstein breeds should not

preventable but intense selection procedure may be helpful in order to reduce the occurrence of urovagina [6].

## CONCLUSION

Urovagina is one of the important conditions causing serious cause of necrotizing endometritis and infertility in dairy animals and mostly reported in Holstein cows. It causes colossal economic loss to the dairy farmers owing to more culling of animals due to infertility. Horizontal vulva with cranioventral displacement of entire reproductive tract due dystocia, relaxation of muscle and ligament in old pluriparous and thin emaciated (BCS<2.5) animals and cows suffered with pneumovagina, cystic ovarian degeneration and endometritis. Vesicovaginal reflex can be effectively diagnosed by vaginoscopy, ultrasonography and rectal examination. Treatment option of urovagina may be conservative or surgical. Invasive surgical correction of urovagina may be necessary when the cow is extremely valuable or when conservative efforts have failed to allow conception but is not economically pragmatic to dairy practice. The most common surgical techniques used to treat affected cows are the transverse fold technique, a modified McKinnon technique, and a modified Brown technique. Transverse fold technique is laborious and may interfere with coitus and in modified McKinnon or a modified Brown technique fistula formation is the major drawback. Recently vestibulovaginal cerclage technique has effectively used to treat the vesicovaginal reflex and it could dramatically increase the fertility of cows. The ozone flush coupled with intracornual insemination is a novel, less-invasive and more practical treatment modality that leads to successful conceptions and pregnancies in dairy cows.

## REFERENCES

- [1] Lopez-Gatius, F.: *Theriogenology*, 60: 89-99 (2003).
- [2] Lucy, M.C.: *J. Dairy Sci.*, 84: 1277-1293 (2001).
- [3] Jean, G.S., Hull, B.L., Robertson, J.T., Hoffsis, G.F. and Haibel, G.K.: *Vet. Surg.*, 17: 258-262 (1988).
- [4] Gilbert, R.O., Wilson, D.G., Levine, S.A. and Bosu, W.T.: *J. Am. Vet. Med. Assoc.*, 194: 931-932 (1989).
- [5] Youngquist, R.S.: *Current therapy in large animal Theriogenology*. 1<sup>st</sup> Edn. W.B. Saunders (1997).
- [6] Noakes, D.E., Parkinson, T.J. and England, C.W.: *Veterinary Reproduction and Obstetrics*. Saunders Elsevier Ltd, 406 - 407, 611- 612 (2009).
- [7] Gautam, G., Nakao, T., Koike, K., Long, S.T., Yusuf, M., Ranasinghe, R.M.S.B.K. and Hayashi, A.: *Theriogenology*, 73: 168-179 (2010).
- [8] Gautam, G. and Nakao, T.: *Theriogenology*, 71: 1451-1461 (2009).
- [9] Roberts, S.J.: *Veterinary Obstetrics and Genital Diseases*. 2<sup>nd</sup> Edn. Indian Edition, CBS Publishers, New Delhi (1986).
- [10] Fairyhood, M., Nowrouzian, I., Hovareshti, P., Bolourchi, M. and Nadalian, M.G.: *Iranian J. Preventive Vet. Med.*, 46: 143-148 (2000).
- [11] Hudson, R.S.: *Genital Surgery of the Cow*. In: *Current Therapy in Theriogenology* (Morrow, D.A. ed.). Vol. 2. WB Saunders, (1986).
- [12] Drivers, T.: *Rebhun's Diseases of Dairy Cattle*, 2<sup>nd</sup> Edn. Saunders/Elsevier Ltd, 421- 422 (2008).
- [13] Gonzalez-Martin, J.V., Astiz, S., Elvira, L. and Lopez-Gatius, F.: *Theriogenology*, 69: 360-365 (2008).
- [14] Brown, M.P., Colahan, P.T. and Hawkins, D.L.: *J. Am. Vet. Med. Assoc.*, 173: 1005-1007 (1978).
- [15] Monin, T.: *Vaginoplasty: A surgical treatment for urine pooling in mare*. In: *Proceedings of 18th American Association of Equine Practice* (1972).
- [16] Easley, K.J.: *Veterinary Clinics of North America: Equine Practice*, 4: 407-416 (1988).
- [17] Zobel, R., Tkalcic, S., Stokovic, I., Pipal, I. and Buic, V.: *Reprod. Dom. Anim.*, 47(2): 293-298 (2012).
- [18] McKinnon, A.O. and Belden, J.O.: *J. Am. Vet. Med. Assoc.*, 192: 647-650 (1988).
- [19] Fubini, S.L. and Ducharme, N.G.: *Farm Animal Surgery*. 1<sup>st</sup> Edn. Saunders/Elsevier, 390 -394 (2004).
- [20] Wolfe, D.F. and Moll, H.D.: *Large Animal Urogenital Surgery*. 2<sup>nd</sup> Edn. Williams and Wilkins (1999).
- [21] Prado, T.M., Schumacher, J., Hayden, S.S., Donnell, R.L. and Rohrabacher, B.W.: *Theriogenology*, 67: 1512-1517 (2007).