STUDY ON UROVAGINA VIS-À-VIS REPRODUCTION IN COWS

THESIS

BY

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Submitted to

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Dedicated to

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CERTIFICATE- I

This is to certify that the thesis entitled **"Study on urovagina vis-à-vis reproduction in cows"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the discipline of **Animal Reproduction, Gynaecology and Obstetrics** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Subrahmanyam Vatasyan** (**V-2009-30-14**) son of Sh. Leeladhar Vatasyan under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

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CERTIFICATE- II

This is to certify that the thesis entitled **"Study on urovagina vis-à-vis reproduction in cows"** submitted by **Subrahmanyam Vatasyan** (**V-2009-30-14**) son of Sh. Leeladhar Vatasyan to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the subject of **Animal Reproduction, Gynaecology and Obstetrics** has been approved by the Advisory committee after an oral examination of the student in collaboration with an External Examiner.

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CONTENTS

ABBREVIATIONS USED

et al.	:	et alli (and others)
%	:	Per cent
sec.	:	Seconds
d	:	Days
mm	:	Millimetre
cm	:	Centimetre
ed.	:	Editor
eds.	:	Editors
mg	:	Milligram
i.m.	:	Intra-muscular

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ABSTRACT

Optimum cattle reproduction is a pre-requisite for a successful dairy enterprise. Urovagina is the pooling of urine in the cranial portion of vagina, which leads to infertility in the affected cattle and therefore economic loss. Studies on urovagina in Indian cattle were completely lacking and therefore undertaken in the Jersey upgraded cows.

In the first part, 31 cows were examined at monthly interval from third month of gestation till second month postpartum for presence of urovagina and change in angles of pelvic girdle, vagina and vulva. There was no evidence of urovagina and no significant difference in the angles of pelvic girdle and vagina at different pregnant and postpartum stages. However, the vulvar angles of 21.6 ± 1.2 and 20.2 ± 0.8 at fourth and seventh month of pregnancy, respectively, were higher ($P<0.05$) than 17.3 ± 0.7 at first month after calving. This difference of approximately three degrees was inferred to be too small for any practical significance. The correlation matrix revealed a significant and individual association among pelvic girdle, vagina and vulva.

In the second part, 30 normal and 15 urovagina cows were examined for vaginoscopy (to detect the presence and severity of urovagina), nature of vaginal contents, function of constrictor vestibuli muscle, past and current reproductive status, age, parity, body condition score, angles (pelvic girdle, vagina and vulva), vaginal and uterine biopsies and blood serum calcium. Except for the angles that were determined both at Day 0 (estrus) and Day 14 (two weeks after end of estrus), all other investigations were carried out at Day 0. Vaginoscopy confirmed the presence of urovagina in the affected cows and moderate urovagina (n = 8) was most common followed by severe (n = 6) and mild urovagina (n = 1). The contents in vaginal fornix of the affected cows confirmed the presence of urine mixed genital secretions characterised by yellow colour, watery spinbarkeit, 107.6 ± 10.5 ml volume, uremic odour and an average pH of 8.8 ± 0.01compared to normal cows having a clear, copious and stringy genital discharge with 17.2 ± 0.3 ml volume and an average pH of 7.01 ± 0.01. The later two were significantly different from the normal cows. The other significant differences ($P<0.05$ atleast) between the urovagina and the normal cows included (i) longer estrous duration (2.8 ± 0.3d versus 1.1 ± 0.05 d) (ii) higher parity (3.8 ± 0.5 versus 2.4 ± 0.3) (iii) more cranioventral angles of pelvis at Day 0 (0.3 ± 5.4 versus 15.7 ± 0.5) and Day 14 (-15.8 ± 2.8 versus 14.0 ± 1.3), respectively. The other revelations in the urovagina cows were histopathological confirmation of vaginal smooth muscle degeneration around external urethral orifice and endometritis. Surgical correction by creating vestibulovaginal cerclage was attempted 12d after estrus in five urovagina cows. There was complete absence of urine pooling in three of the treated cows indicating a 60% efficacy of the surgical treatment.

In conclusion, prolonged estrous duration in multiparous cows having a craniovental pelvic girdle and degenerating vaginal smooth muscles around external urethral orifice increase the likelihood of urovagina. Vestibulovaginal cerclage will be efficient to resolve urovagina in cows.

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(Signature of the student with date) (Signature of Major Advisor)

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INTRODUCTION

Chapter 1

INTRODUCTION

In recent years, the main emphasis of developments in bovine reproductive technology has been to preserve fertility. Repeat breeding on the other side, having a multifactorious etiology, reduces the fertility and profitability of a dairy enterprise. Certain anatomical derangements of the female reproductive tract, either by themselves or in association with the urinary tract, have been linked to reproduction losses in cows. One such condition is urovagina. Also known as vesicovaginal reflux, urovagina refers to accumulation of urine in the cranial portion of vagina and is associated with infertility in cows (Hudson 1986; St. Jean *et al*. 1988; Gilbert *et al.* 1989; Youngquist 1997; Fubini 2004) and mares (Monin 1973; Easley 1988).

Amongst cattle, urovagina has been mostly reported in Holstein and Charolais (Noakes *et al*. 2009). The prevalence of urovagina in two separate studies has been 1.5 to 11.3% in Holstein cows (Farhoodi *et al*. 2000; Gautum and Nakao 2009). Urovagina is more commonly noticed in old pluriparous cows (St. Jean *et al*. 1988; Hooper and Taylor 1995; Fubini 2004; Prado *et al.* 2007) and mares (Zemjanis 1970; Lieux 1972; Monin 1973; Vaughn 1974; Thornbury 1975). Increase in age and parity leads to cranioventral displacemet of pelvis (Fubini 2004; Prado *et al*. 2007) as well as uterus and vagina (St. Jean *et al*. 1988; Gonzalez-Martin *et al.* 2008; Ewoldt 2009). The displacement of uterus and vagina may also be linked to damage of constrictor vestibuli muscle (St. Jean *et al*. 1988; Gonzalez-Martin *et al*. 2008) which, besides holding uterus and vagina in taut (Easley 1988; St. Jean *et al*. 1988) also regulates the functioning of urethra and external urethral sphincter in urinary continence (Reece 2004). The displacement of pelvis and damage to constrictor vestibuli muscle are common sequel to violent struggling during parturition, excessive fetal traction during vaginal delivery of fetus in dystocia and during prolapse (Cox 1987; St. Jean *et al*. 1988; Wolfe and Baird 1993; Hooper and Taylor 1995; Fubini 2004; Gonzalez-Martin *et al.* 2008). In any case, a change in orientation of the aforesaid genital and associated musculoskeletal components raises the dorsally positioned external urethral orifice in relation to the cranial portion of vagina, which during micturition results in gravitation of urine in the vaginal fornix (Hudson 1986; Cox 1987; St. Jean *et al*. 1988; Gilbert *et al*. 1989; Fubini 2004; Prado *et al*. 2007). Low body condition score has been

indicated as another significant risk factor associated with urovagina (Gautum and Nakao 2009).

The collection of urine in the fornix vagina induces a sac formation and aggravates the drooping of vagina in abdomen. Depending on the volume of urine in fornix, the cervical external-os may be partially or completely dipped (Gautum and Nakao 2009). Upto 3.5 l of urine has been recorded in vaginal fornix of some affected cows (Hudson 1986). Dipping of cervix in urovagina contents allows the urine to even enter into the uterus (Hudson 1986). Presence of urine in the genital tract induces a varying degree of localized vaginitis, cervicitis and endometritis (Monin 1973). In the affected cows, the pH of genital and urinary contents in vaginal fornix increases abnormally to >7.4 (Gautum and Nakao 2009). Resultantly, the genital environment in the urovagina cows becomes hostile for sperm and embryonic survival leading to infertility (Hudson 1986; Gilbert *et al*. 1989; Wolfe and Baird 1993; Youngquist 1997). Cows with persistent urovagina required more services per conception and had more days open (Gautum and Nakao 2009).

Spontaneous recovery from mild urovagina in cows is on record (Gautum and Nakao 2009), but persistent and more severe cases require surgical intervention (St. Jean *et al*.1988; Gautum and Nakao 2009). Amongst the three different surgical treatments comprising of transverse fold technique, urethral extension and vestibulovaginal cerclage, the later appears to be least invasive and most promising (Gonzalez-Martin *et al.* 2008).

Jersey upgraded cows is the most important cattle breed recommended for the hilly regions of India, including Himachal Pradesh (Banerjee 2009). Crossbred cows have been indicated to more vulnerable to different diseases (Deshmukh and Kaikini 1999) as also confirmed by a recent study suggesting Jersey crossbred to be more vulnerable to prolapse and abnormal birth (Singh *et al*. 2008). However, there is no Indian study on urovagina in cows.

The present study was undertaken in the Jersey upgraded cows with the following objectives:

1. To investigate the cows under different stages of reproduction for detection of urovagina.
2. To treat urovagina in cows.

REVIEW OF LITERATURE

Chapter 2

REVIEW OF LITERATURE

Normal fertility indices are essential to meet the ever increasing demand of milk production and normal calf-crop cycle in cows. Infertility due to managemental problems, functional disturbances and infectious reasons has been the major concern of different workers (Gilbert *et al.* 2005; Yusuf *et al*. 2010). However, several other less prevalent reasons of reproduction failure have acquired importance in the recent past due to a reason that as reproductive performance of an individual cow assume importance in a dairy enterprise.

Rectovaginal injuries cause different kinds of abnormalities in reproductive tract, which are serious enough to cause infertility and economic loss (Dreyfuss *et al.* 1990). Rectovaginal fistula, third degree perineal laceration, pneumovagina, urovagina and mixed injuries are the different consequences to rectovaginal injuries (Dreyfuss *et al.* 1990). In a herd of 1420 Holstein cows in Tehran, rectovaginal injuries were recorded in 10.3% cows. In descending order of predominance, pneumovagina, third degree laceration, urovagina, mixed injuries and rectovaginal fistula were observed in 5.7, 1.9, 1.5, 0.8 and 0.4%, respectively. Importantly urovagina had the highest recurrence risk of 14% followed by 11% of third degree lacerations (Farhoodi *et al.* 2000).

Urovagina/vesicovaginal reflux, or the pooling of urine in cranial vagina was initially reported in mares (Monin 1973) and subsequently in cattle (St. Jean *et al.* 1988)

2.1 Prevalence

Available literature indicates a preponderance of urovagina in Charolais and Holstein breeds of cattle (Noakes *et al.* 2009). In an earlier study, urovagina was prevalent in 1.5% of 1420 Holstein cows raised in Tehran (Farhoodi *et al.* 2000). A later study, however, reported a much higher prevalence of 26.7% during 344 lactations in 250 Holstein cows in Japan (Gautum and Nakao 2009).

2.2 Etiology

2.2.1 Age and parity

Urovagina is more common in older and multiparous cows (St. Jean *et al.* 1988; Hooper and Taylor 1995; Fubini 2004; Prado *et al.* 2007), which is attributed to a change in anatomical conformation and/or relationship between uterus, cervix, vagina, vestibule, vulva, anus, external urethral orifice and pelvic girdle. Aged cows

with higher number of calvings have increased abdominal girth and weakened abdominal musculature, which increases the tension on anus causing it to be 'sunken' (Pauret 1982). The anal sphincter, constrictor vulvae muscle and constrictor vestibuli muscle have a close anatomical association amongst them in cows (St. Jean *et al.* 1988) and mares (Easley 1988). Hence, the sinking of anus also distorts the conformation of vagina and vestibule causing them to be displaced cranially and ventrally in cows (St. Jean *et al.* 1988) and mares (Easley 1988).

Vagina normally slopes ventrally and anteriorly (Camp 1986). An increase in vaginal slope exaggerates and prevents complete evacuation of urine from vestibule (Camp 1986). Increased vaginal slope has been linked to cranial advancement of uterus and cervix into the abdominal cavity due to more stretched supporting ligaments in multiparous cows (St. Jean et al. 1988; Fubini 2004; Noakes *et al*. 2009) and mares (Camp 1986). Increase in vaginal angle and cranioventral tipping of pelvis (downward and forward movement of tuber coxae), common in old cows, raises the external urethral orifice in relation to the vaginal floor, leading to pooling of urine in the vaginal fornix (Prado *et al*. 2007)

2.3 Angulations

2.3.1 Pelvic girdle

Congenital or acquired cranioventral tipping of pelvis increases the chances of urovagina in cows (Hudson 1986) and mares (Brown *et al.* 1978). Cows with cranioventral tipping of pelvis had a higher prevalence of urovagina compared to the ones with a caudoventral pelvis (Gautum and Nakao 2009). Cranioventral pelvic girdle causes the external urethral orifice to be positioned at a relatively higher level in the vestibule, which increases the tendency of urine pooling in the vaginal fornix (Hudson 1986; Cox 1987; St. Jean *et al*. 1988; Fubini 2004; Prado *et al*. 2007).

2.3.2 Vagina

Amongst different segments of tubular genital tract, the contour of vagina makes it most vulnerable for an anatomical change in the presence of an exciting cause. Vagina normally slopes anteriorly and ventrally (Camp 1986). However, increase in the vaginal slope in anterior and ventral direction favours urovagina (Hudson 1986; Gonzalez-Martin 2008). The vagina falls below the level of pelvic floor due to age (St. Jean *et al.* 1988) and due to loose vaginal walls because of poor body condition in cows (Gautum and Nakao 2009) and due to poor vaginal musculature and elongated ovarian ligament in mares (Zemzanis 1970; Lieux 1972;

Monin 1973; Vaughn 1974; Thornbury 1975). The pooling of urine further gravitates the cranial vagina and aggravates urovagina (Hudson 1986).

2.3.3 Vulva

Urovagina was more common in the cows with an abnormally sloping vulva (Ewoldt 2009). In normal cows the vulvar lips are angled <45° to the vertical line and such vulva has been referred to as a 'vertical vulva' (Gautum and Nakao 2009). However, cows with vulvar lips angled >45° to the vertical line, referred to as a 'horizontal vulva' (Gautum and Nakao 2009) are nine times more likely to suffer from urovagina compared to cows with a vertical vulva (St. Jean *et al*. 1988; Gautum and Nakao 2009). Sunken anus (St. Jean *et al*. 1988) and cranial displacement of uterus, cervix and vagina (St. Jean et al. 1988; Gonzalez-Martin *et al*. 2008) may pressurize the vulva to be displaced in a horizontal manner. A horizontal vulva may favour introduction of faecal material, urine and air into vagina (Roberts 1986; Cox 1987; Riketts 1991; Gautum and Nakao 2009).

2.4 Constrictor vestibuli muscle

Constrictor vestibuli is a striated muscle made up of circular and longitudinal fibres. It innervates the outer wall of vestibular region, is incomplete dorsally and passes ventrally into the urethral muscle. The smooth muscle inside the constrictor is a continuation of the vaginal musculature, which intermixes with the striated muscle in the urethral segment in the ventral vaginal region (Dellman and Brown 1981; Trautmann and Fiebiger 2002).

Dysfunction of constrictor vestibuli muscle due to its damage during normal or assisted parturition favours urovagina (St. Jean et al. 1988; Wolfe and Baird 1993; Hooper and Taylor 1995; Farhoodi et al. 2000; Fubini 2004). The constrictor vestibuli assists in the expulsion of urine from the external urethral orifice. Slackness in the closure of external urethral orifice upon stimulation is suggestive of dysfunction of the constrictor vestibuli muscle (Gonzalez-Martin *et al*. 2008). In addition, the damaged constrictor vestibuli muscle alike the cranioventral tipping of pelvis and vaginal angulation, also raises the external urethral orifice to cause urovagina (Gilbert *et al*. 1989)

2.5 Hormonal influences

Urovagina has also been recognised in heifers that have been repeatedly superovulated in embryo transfer programme (Wenzel and Baird 1998).

Superovulation or the hormones used in this process contribute to pelvic ligament laxity, which can lead to cranioventral displacement of vagina (Fubini 2004) and resultantly urovagina (Camp 1986).

2.6 Effect of urovagina on genital tract

The pooling of increasing volume of urine in the anterior part of vaginal vault induces sac formation and eventual drooping of the dilated vaginal vault into the abdominal cavity (Hudson 1986). The urine and vaginal debris provoke cervicitis and these may even enter the uterus during estrus to cause superficial (Monin 1973) or necrotizing (St. Jean *et al*. 1988) endometritis. Urine induces local pH changes in the genital tract which then becomes spermicidal to cause fertilization failure and even provoke early embryonic death (Hudson 1986; Gilbert *et al*. 1989; Wolfe and Baird 1993; Youngquist 1997)

2.6.1 Severity of urovagina and infertility

Depending on the extent of covering of the external-os of cervix by urine or urine mixed mucus, urovagina was classified into three categories (Gautum and Nakao 2009) as follows:

(i) **Mild urovagina**: Presence of small amount of urine or urine mixed mucus (10-100 ml) on the floor of vagina but not covering the external-os of cervix (no risk of urine entering into uterus)

(ii) **Moderate urovagina**: Presence of appreciable amount of urine or urine mixed mucus (100-500 ml) covering up to half of the external-os of cervix (some risk of urine entering into the uterus)

(iii) **Severe urovagina**: Presence of large amount of urine or urine mixed mucus (>500 ml) covering at least half (up to entire) external-os of cervix (high risk of urine entering into the vagina)

Another report indicated presence of up to 3.5 L urine in the vaginal fornix of the affected cattle (Hudson 1986).

The percentage of mild, moderate and severe urovagina in the affected cows was 42.4, 41.3 and 16.3%, respectively. Moderate and severe urovagina are more likely to disrupt reproduction (reduce pregnancy rate by 53% per insemination) and were therefore designated as clinically relevant urovagina (Gautum and Nakao 2009).

2.7 Detection of urovagina

Urovagina in cows is mostly detected at estrus when transrectal manipulation of genitalia reveals a gush of abnormally large quantity of watery genital discharge.

Transrectal palpation of reproductive tract can detect urine pooling in severe cases of urovagina (St. Jean *et al*. 1988), whereas moderate cases of urovagina may go unnoticed. Hence, vaginoscopy remains a practical tool to diagnose urovagina (Fubini 2004) and to determine if surgical correction of urovagina is required (Gautum and Nakao 2009).

2.7.1 Examination of vaginal contents

Urine pooling must be differentiated from the serous mucus that occasionally collects posterior to the cervix during estrus (Camp 1986). Cows with vaginal contents that are yellowish in colour, watery in consistency, uremic in smell and have a pH value of more than 7.4 are said to be suffering from urovagina (Gautum and Nakao 2009). If confusion still exists regarding the nature of accumulated fluid in vagina, laboratory examination for calcium carbonate crystals, creatinine or urea nitrogen may be helpful (Trotter 1992).

2.7.2 Histopathology

A uterine biopsy is useful for determining the degree of damage to the uterine wall (Fubini 2004). In normal cows, the uterine endometrium during estrus is edematous and metrorrhagic (Ohtani *et al*. 1993). A few polymorphonuclear leukocytes invade lamina propria, luminal epithelium and uterine lumen. The endometrial glands are enlarged and edematous with more tortuous crypts (Shukla 1988; Lohuis *et al.* 1992).

Histological examination of an endometrial specimen from an endometritis cow revealed a marked neutrophillic infiltration, which is often related to infertility (Studer and Morrow 1978). The necrotizing endometritis is characterized by sloughing of the luminal epithelium and infiltration of the stratum compactum with numerous neutrophills and a few macrophages (Gilbert *et al.* 1989). A subacute endometritis is characterized by varying degree of glandular epithelial degeneration along with cellular debris in the lumen of cystic gland (Shulka 1988; Sawamukai 1994).

2.8 Treatment of urovagina

2.8.1 Evacuation of vaginal contents

In this form of treatment, cervix is drawn back caudally and dorsally several times by transrectal manipulation to express air and urine from vagina. The procedure needs to be repeated two or three times a week. A total of 25 cows were treated in this manner, all recovered and were bred (Lean 1990)

2.8.2 Surgical correction

Surgical correction has been recommended when the vaginal content of urine exceeds 100 ml (St. Jean *et al*. 1988). Surgical correction for urovagina in cows and mares has been separated into three categories: (i) vaginoplasty (ii) extension of the urethra (iii) vestibulovaginal cerclage.

In vaginoplasty, also referred as transeverse fold technique, the folds of vaginal mucosa are elevated to create more prominent transverse folds that act as a dam and are held in place by a series of mattress sutures. This technique, used in mares, does not function well in cattle (Fubini 2004). Moreover, the long term success rates of vagnioplasty in mares are unavailable (Wenzel and Baird 1998; Fubini 2004). In urethral extension technique, the functional urethra is extended caudally by creating a tube of vaginal mucosa (Wenzel and Baird 1998). This technique resolved urovagina in a single cow (Gilbert *et al.* 1989) and 88.8% (16/18) mares (Brown *et al*. 1978). In vestibulovaginal cerclage method, the cerclage of vestibulovaginal junction (Plate 4.5) under the vaginal wall (submucosal) cranial to urethral opening prevented retrograde flow of urine into vagina in cattle. The technique was effective in impregnating 74% (14/19) affected cows (Gonzalez-Martin *et al.* 2008).

MATERIALS AND METHODS

Chapter 3

MATERIALS AND METHODS

3.1 Animals and place of work

The present study was conducted in a total of 76 Jersey crossbred cows. The details of the cows were as follows:

(a) Sixty one cows, 31 pregnant and 30 non-pregnant and cyclic cows without urovagina (hereafter mentioned as normal cows) belonged to the Livestock Dairy Farm of Himachal Pradesh Krishi Vishvavidyalaya, Palampur.

(b) Fifteen cyclic urovagina (hereafter mentioned as urovagina cows) were from different locations in the nearby areas. The cows were said to have urovagina if the contents of vaginal fornix were characterised by- abnormally higher volume, watery spinbarkeit, urine like odour and colour and pH >7.4 (Gautum and Nakao 2009).

The pregnant cows were examined at monthly interval from third month of gestation till second month postpartum. Each normal and urovagina cow was examined on the day of estrus (Day 0) and fourteen days after end of estrus (Day 14).

3.2 List of different examinations.

The details of different investigations/examinations conducted on different cows are presented in Table 3.1.

Information on reproductive performance, age and parity was based on the available records in normal cows and history from the owner of urovagina cow.

3.2.1 Vaginoscopy

This was performed by inserting a sterile and lubricated vaginal speculum to ascertain the presence of genital secretions and/or urine in the fornix vagina. Adequate illumination source was used to visualise the vaginal fornix.

3.2.2 Severity of urovagina

The extent of dipping of external-os in the pool of accumulated contents in vaginal fornix was recorded and classified as follows (Gautum and Nakao 2009):

(a) Mild urovagina: small amount of urine or urine-mixed mucus only on the floor of the vagina, but not covering the external-os of cervix.

(b) Moderate urovagiana: appreciable amount of urine or urine-mixed mucus covering upto half of the external-os of cervix (some risk of urine entering into the uterus).

Table 3.1 List of different observations/investigations performed (√) or not performed (×) in the pregnant-postpartum, normal and urovagina cows.

Type of cows / Investigations		Pregnant-postpartum[1] (n=31)	Cyclic	
			Normal [2] (n=30)	Urovagina [2] (n=15)
1	Vaginoscopy	√	√	√
2	Severity of urovagina	×	√	√
3	Examination of vaginal contents (colour, spinbarkeit, volume, pH, odour)	×	√	√
4	Functional status of constrictor vestibuli muscle	×	√	√
5	Reproductive status	×	√	√
6	Age, parity and Body condition score (BCS)	×	√	√
7	Angles			
	(i) Pelvic girdle	√	√	√
	(ii)Vagina	√	√	√
	(iii)Vulva	√	√	√
8	Biopsy			
	(i)Vaginal	×	√	√
	(ii)Uterine endometrium	×	√	√
9	Blood serum	×	√	√
10	Surgery	×	×	√ [3]

1. The listed examinations at monthly interval
2. The listed examinations at Day 0, except for angles recorded also at Day 14

3. At Day 12 after end of estrus (n=5)

(c) Severe urovagina: large amount of urine or urine-mixed mucus covering at least half upto the entire external-os of cervix (high risk of urine entering into the uterus).

3.2.3 Examination of vaginal contents

After vaginoscopy, the fornix vagina was evacuated through transrectal manipulations. The contents were collected in a clean, wide pan and characterized for colour, spinbarkeit, volume, odour and pH. The colour was classified as clear or yellowish. The spinbarkeit was classified as stringy or watery. The volume was estimated in a measuring cylinder. The contents were recorded for the presence/absence of urine like odour. The pH was evaluated by a pH paper detecting pH in range of 2 to 10 and classified as pH<7 or pH>7.

3.2.4 Functional status of constrictor vestibule muscle

It was evaluated by recording the time taken for normal closure of the external urethral orifice after removing the little finger inserted in the external urethral meatus. Time of <1 second was considered as normal, whereas time of >1 second was considered as impaired function of the said muscle (Gonzalez-Martin *et al*. 2008).

3.2.5 Reproductive status

The reproductive assessment was based on number of inseminations, interestrus interval, estrous duration and any treatment given to the cow since last calving. The past history for an evidence of postpartum problems (dystocia and prolapse) was also recorded.

3.2.6 Age, parity and BCS

After recording age and parity, the BCS was recorded on a scale of 1.0 to 5.0 with an increment of 0.25 (Edmondson *et al.* 1989).

3.2.7 Angles

3.2.7.1 Pelvic girdle

To ascertain the angle of pelvic girdle, a long rigid wooden scale was placed from the middle of tuber ischii towards tuber coxae in a horizontal axis (Line A). Another wooden rod was placed in a manner that it connected middle of tuber ischii and tuber coxae (Line B). The angle formed at the convergence of Line A and Line B, considered as Point C, was spanned with a divider and measured on a protector. Line A, Line B and Point C are depicted in Plates 3.1 and 3.2, respectively.

The tuber coxae was assumed to be the cranial part of pelvis. Hence, the displacement of pelvic girdle was described on the basis of dorsal or ventral shift of tuber coxae from the horizontal axis. If the tuber coxae was at a higher level than the tuber ischii, the angle of pelvic girdle increased (positive value) and was termed as being craniodorsal (Plate 3.1). However, if tuber ischii was at a higher level than tuber coxae, the angle of pelvic girdle decreased (negative value) and was termed as being cranioventral (Plate 3.2).

3.2.7.2 Vagina

To measure the vaginal angle, a plastic A.I. sheath, was inserted along the floor of vagina till it entered vaginal fornix (Line A). Thereafter, the divider was placed on the A.I. sheath in a manner so that one of its arm was parallel to the sheath (Line A) and the other arm constituted a horizontal axis (Line B).

Fornix was considered the cranial part of vagina. If Line A sloped away from the ground, the vaginal angle reduced (negative value) and was termed as cranioventral vagina (Plate 3.3). However, if the Line A sloped towards the ground, the vaginal angle increased (positive value) and was termed as craniodorsal vagina (Plate 3.4).

3.2.7.3 Vulva

To ascertain the vulvar angle, a divider was placed, in a manner so that one of its arm was in contact with vulvar commissure and other in a vertical axis. The angle so formed between the vulvar commissure and vertical axis constituted the angle of vulva (Plate 3.5).

3.2.8 Collection of biopsies for histopathological examination

Using a sterile Neilson's biopsy punch, biopsies were collected from the uterine endometrium and caudal vagina in the normal and the urovagina cows.

3.2.8.1 Uterus

The uterine biopsy was collected from the dorsolateral part of an uterine horn.

3.2.8.2 Vagina

The vaginal biopsy was collected under posterior epidural analgesia. A well lubricated vaginal speculum was inserted through the vulva to locate the external urethral orifice on the vaginal floor. Thereafter, approximately 5 cm lateral to the external urethral orifice a half inch deep incision was made with the help of a Metzenbaum scissors. Subsequently, a sterile Neilson's Biopsy Punch was inserted to

grasp one of the tissue edge, the punch was then rotated three to five times to remove a piece of tissue.

All the biopsies were preserved after collection in buffered neutral formal saline (10%) till processing for histopathological studies.

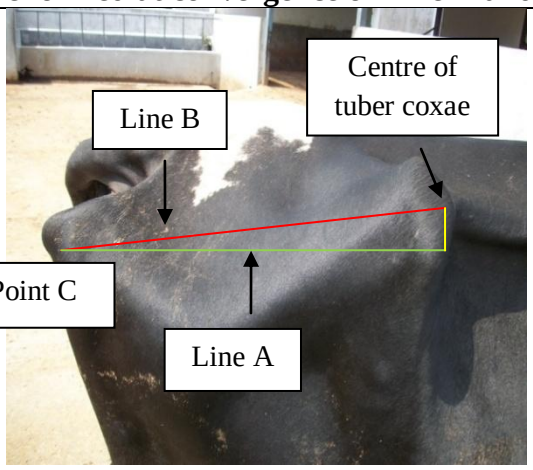
3.2.9 Collection of blood samples

Blood sample was collected from jugular venipuncture in a sterile vial. Serum was harvested for estimation of calcium using standard kits in an automated Blood Chemistry Analyser (RA-5010).

Plate 3.1 Craniodorsal pelvic girdle (note-higher tuber coxae than tuber ischii)

Plate 3.2 Cranioventral pelvic girdle (note-lower tuber coxae than tuber ischii)

Line A: Horizontal axis; Line B: Runs from centre of tuber ischii to tuber coxae; Point C: Angle formed at convergence of Line A and Line B



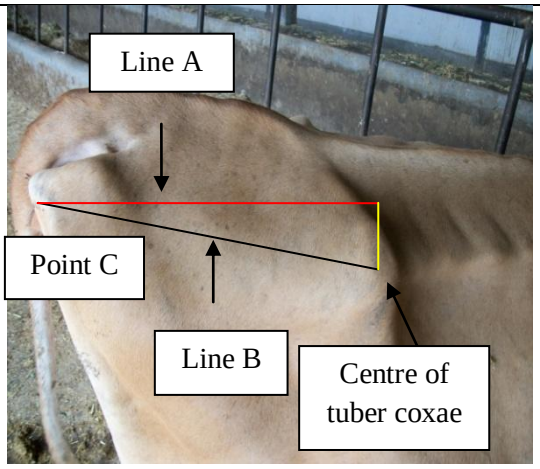
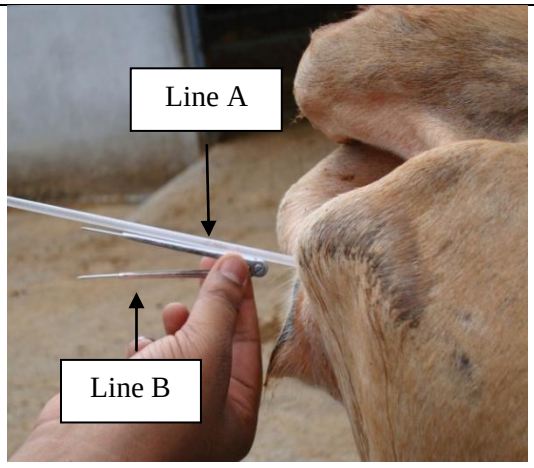


Plate 3.3 Cranioventral vagina

Plate 3.4 Craniodorsal vagina

Line A: Slope of vaginal floor; Line B: The horizontal axis



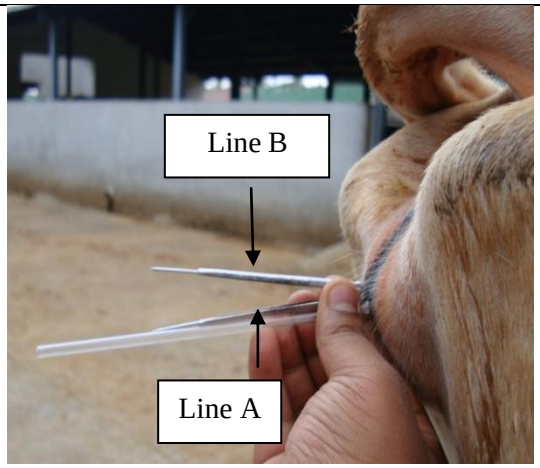
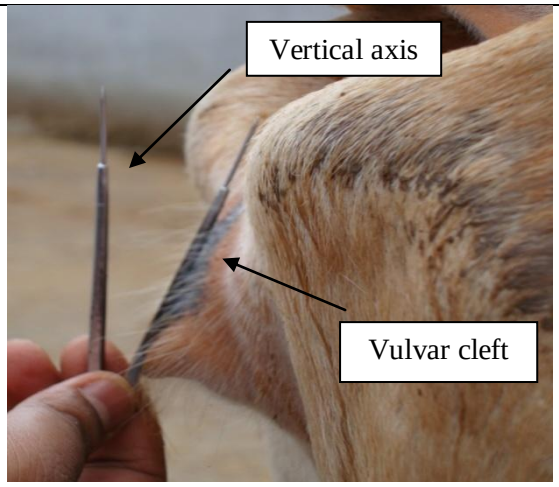


Plate 3.5 Angle of Vulva



3.2.10 Surgical correction of urovagina

The surgical correction of urovagina was undertaken aseptically in five of the urovagina cows on Day 12 after end of estrus using the method of Gonzalez-Martin *et al.* (2008). The surgery was aimed to constrict the vestibulovaginal junction for preventing the retrograde flow and accumulation of urine in the fornix vagina.

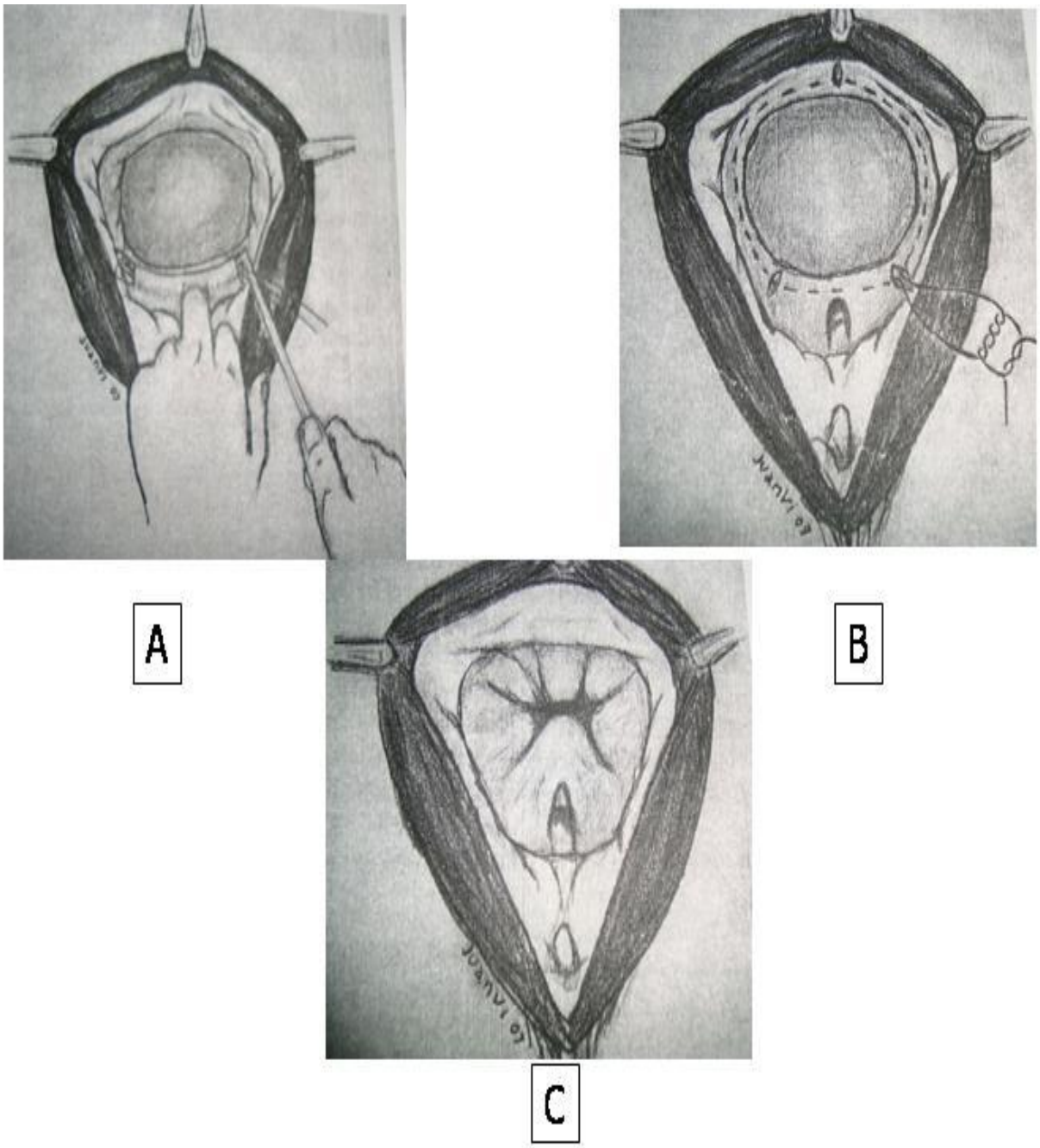
Briefly, the surgical procedure was as follows (Plate 3.6). Under posterior epidural anaesthesia, a 5-10 mm long and 1cm deep incision using Metzenbaum scissors was made at 4 o'clock position in the vestibulovaginal junction. A regular eye, ½ circle, Number 2, cutting needle, threaded with a double bight monofilament polydioxanone violet (PDS II, Ethicon, Johnson and Johnson Limited B-15/1, M.I.D.C., Waley, Aurangabad-431136) was inserted through the incision and passed till it was exposed at 8 o'clock position. The needle was withdrawn and re-entered at 8 o'clock position till it was exposed at 12 o'clock position. The needle was once again withdrawn and the last bite encompassed 12 o'clock area to be finally withdrawn from the 4 o'clock position. The two ends of the suture exiting from the incision at 4 o'clock position were tightened enough to permit the entry of one finger from the circlage. Surgeon's knot was applied and the suture was completely embedded in the mucosa at 4 o'clock position. During the entire surgical process, the vulva and vestibular region were dilated manually using Allies Forceps.

The success of surgery was evaluated by presence of (i) normal defecation and urination by the cow, (ii) one finger constriction and (iii) absence of urine in genital discharge at the subsequent estrus.

3.3 Statistical analysis of data

In pregnant-postpartum cows the Pearson correlation coefficient was determined between the angles of pelvic girdle, vagina and vulva at different gestation and postpartum stages. A similar association was also determined at Day 0 and Day 14, though separately, in the normal and the urovagina cows. In addition, the average age, parity, BCS and estrous duration were compared between the normal and urovagina cows. Regression equation was framed using statistically significant variables in the urovagina cows. For determining statistical significance between two values, a difference of $P < 0.05$, atleast, was considered significant. The entire statistical analysis was performed using Software Package for Social Sciences (SPSS version 16.0).

Plate 3.6 Vesibulovaginal cerclage technique



A: Entry of suture needle at 4 o' clock and exit at 8 o' clock position

B: The points of entry and exit of threaded needle at different positions. Both the ends of thread are embeded submucosal at 4 o' clock position after applying surgeon's knot

C: Vestibulovaginal cerclage with one finger space in centre

RESULTS AND DISCUSSION

Chapter 4

RESULTS AND DISCUSSION

Urovagina refers to the pooling of urine mixed genital secretions in the vaginal fornix (Hudson 1986; St. Jean *et al.* 1988; Gilbert *et al.* 1989). Presence of urine incites local inflammatory and pH changes that perish the spermatozoa and embryo (Hudson 1986; St. Jean *et al*. 1988; Gilbert *et al*. 1989; Wolfe and Baird 1993; Youngquist 1997). The urovagina affected cows have impaired fertility incriminating to economic losses (St. Jean *et al*. 1988; Fubini 2004). The available studies on urovagina in cows confine to Holstein and Charolais which, however, cannot be extrapolated to Jersey crossbred cows.

The present work in Jersey crossbred cows intended to (i) investigate certain risk factors associated with urovagina and (ii) evaluate efficacy of a surgical intervention in resolving urovagina in cows.

4.1 Vaginoscopy

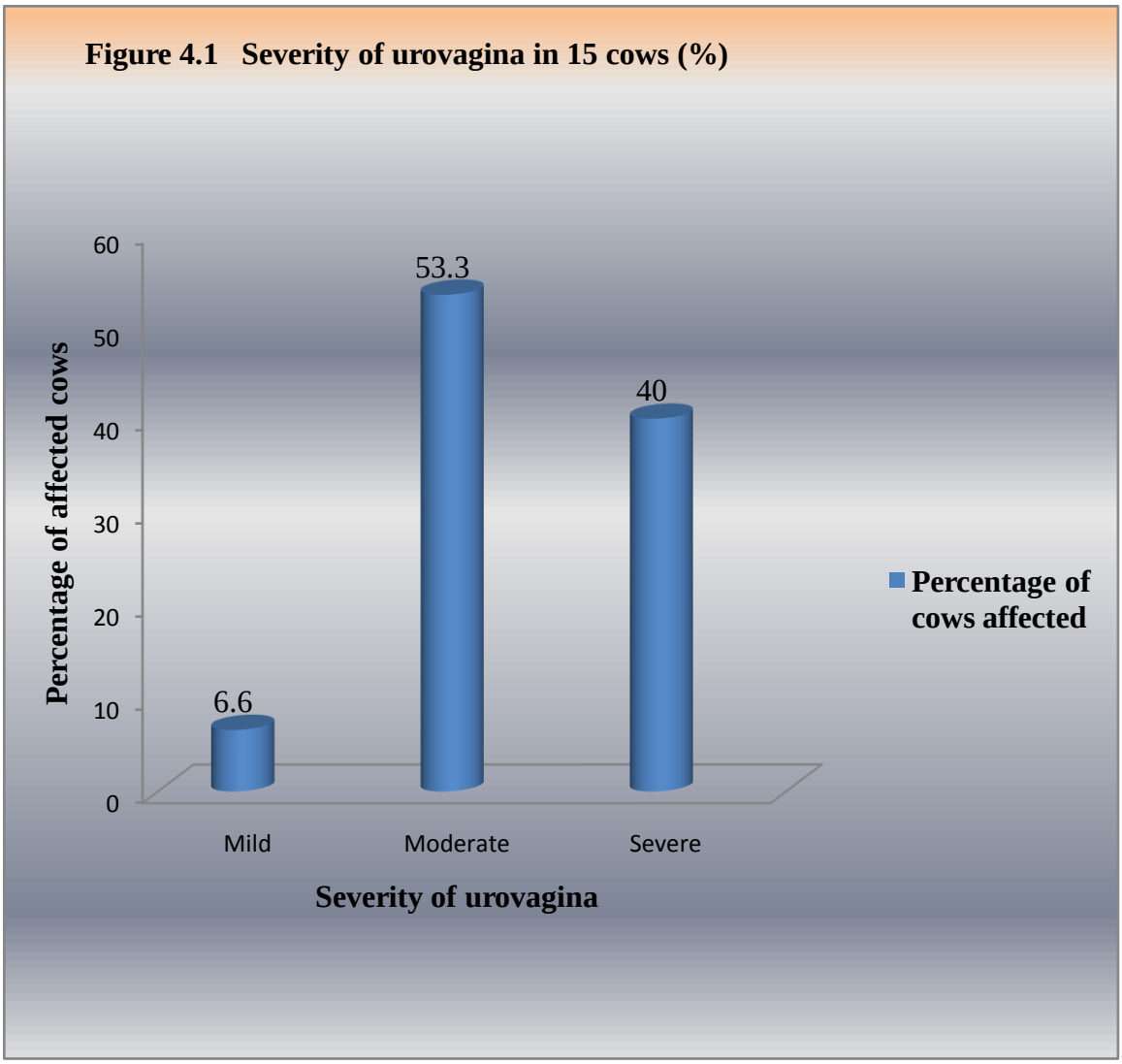
There was no evidence of urovagina during different stages in the pregnant - postpartum as well as at Day 0 in the normal cows. However, transient urovagina has been reported in some cows immediately post partum or during estrus (St. Jean *et al*. 1988). In the present study, the pregnant cows were included to test the hypothesis that advancement in gestation changes the angles of pelvic girdle, vagina and vulva, which may in turn favour urovagina. However, no urovagina in the pregnant cows of present study did not support our hypothesis. There are no previous reports testing presence of urovagina in pregnant cows.

There was pooling of variable amount of urine in the 15 urovagina cows and accordingly a variation in severity (Figure 4.1). The maximum number of urovagina cows had moderate urovagina (n=8; 53.33%) followed by severe (n=6; 40.0%) and mild (n=1; 6.6%) urovagina, respectively. These findings differ from a previous study in 92 urovagina cows that had mild, moderate, and severe urovagina in a descending order of 42.4%, 41.3% and 16.3%, respectively (Gautum and Nakao 2009). A much larger number of cows in the later study could be the possible reason of difference in proportion of urovagina severities from the present study.

4.2 Examination of the vaginal contents

The vaginal contents in all the normal cows at Day 0 were clear, stringy, viscous, 17.2 ± 0.3 ml in volume (range 15-20 ml), 7.01 ± 0.01 in pH (range 7.0-7.2) and had no urine and urine like odour. However, due to the presence of urine and urine like odour the

Figure 4.1 Severity of urovagina in 15 cows (%)



vaginal contents in the urovagina cows were characterized as yellowish, watery, breaking, 107.6 ± 10.5 ml in volume (range 30-200 ml) and with a pH of 8.3 ± 0.01 (range 8.0-9.5). The findings in the urovagina cows of present study are in close agreement to Gautum and Nakao (2009).

4.3 Functional status of constrictor vestibuli muscle

Constrictor vestibuli muscle is a deep skeletal muscle that supplies the urethra and assists in urine expulsion (Dellman and Brown 1981; Trautman and Fiebiger 2002). A failure of the external urethral orifice to close sufficiently in response to insertion of little finger (more than 1 sec to close) indicates lack of integrity and functioning of the constrictor vestibuli muscle, as was recorded in all the 19 urovagina cows investigated elsewhere (Gonzalez- Martin *et al*. 2008). However, a normal closure of external urethral orifice within 1 sec after insertion of little finger rules out

abnormality of constrictor vestibuli muscle in all the urovagina cow of the present study.

4.4 Current reproductive status

Normal cows had no evidence of repeat breeding, but all the urovagina cows were inseminated during more than three consecutive estrous periods since last calving. This finding is in accordance with Gautum and Nakao (2009) who reported that urovagina cows require more inseminations per pregnancy and have more days open. A major finding of the present study was that all the urovagina cows had prolonged estrus of 2.8 ± 0.7 d (range 2 - 4 d) compared to a significantly short estrous duration of 1.1 ± 0.05 d (range 1 - 2 d) in the normal cows. Amongst the 15 urovagina cows, estrous duration of two, three and four days were recorded in five, eight and two cows, respectively. However, in normal cows except for estrous duration of two days in three out of thirty cows, all other cows had estrus lasting one day. There is no available report on estrous duration in urovagina cows. However, Easley (1988) reported that some mares only pool urine during estrus when the reproductive tract is relaxed under the prolonged influence of estrogen. Whether this holds true in the urovagina cows having prolonged estrus in the present study, needs to be validated. Endometritis was a consistent feature in all the urovagina cows of present study (Section 4.9.2). Experimental endotoxin exposure during estrus induced a decreased or delayed LH surge leading to delayed ovulation in one third of the experimental cows (Lavon *et al*. 2008). Although the profile of infectious agents was not determined in the present study, but the endotoxin – LH surge cause – effect relationship leading to prolonged estrus in the urovagina cows of present study, cannot be precluded. Urovagina cows in the present study had no history of dystocia or prolapse.

Pooling of urine in the vaginal fornix either causes or accentuates pre-existing vaginitis or cervicitis to decrease pregnancy rates in the affected cows. The postulated mechanisms to reduce fertility include urine induced pH changes affecting sperm viability and / or endometritis in cows (Hudson 1986; Gilbert *et al*. 1989; Wolfe and Baird 1993; Youngquist 1997) and mares (Monin 1973) or endometritis induced premature lysis of corpus luteum in mares (Easley 1988). Persistence of urovagina also causes periglandular fibrosis in cows (Gilbert *et al.* 1989). Study of the reasons mentioned *vide supra* are incriminated for repeat breeding in all the urovagina cows of present study that failed to conceive after repeated inseminations and even after 5ml

i.m. administration of Receptal® VET (Intervet India Private Limited, each ml contain 0.0042 mg of Buserelin-Acetate) as a treatment of prolonged estrus in some cows.

4.5 Age, parity and BCS

Table 4.01 represents the characterstics of age, parity and BCS of the normal and the urovagina cows. There was no difference in the average age and BCS between normal and urovagina cows. However, the average parity of 3.8 ± 0.5 in the urovagina cows was significantly higher ($P<0.001$) than 2.4 ± 0.3 in the normal cows.

Increase in parity has been an inciting cause of urovagina in cows (St. Jean *et al.* 1988; Fubini 2004) and mares (Pouret 1982; Easely 1988). Parity increase is associated with increased abdominal girth, weakened abdominal musculature and increased tension of anus, resulting in cranial displacement of the later. Sunken anus may be, therefore, an important indication of presence of urovagina in mares (Easley 1988) and cows (St. Jean *et al*. 1988).

Also with increase in parity there is repeated and excessive stretching of constrictor vestibuli and constrictor vulvae muscle during calving, which is accentuated by dystocia (St. Jean *et al.* 1988; Hooper and Taylor 1995; Fubini 2004). This causes dorsal positioning of external urethral orifice to the cranial portion of vagina (Prado *et al*. 2007) alongwith ventral displacement of vagina and vestibule (Easley 1988; St. Jean *et al*. 1988), all of which favour urovagina (Brown *et al*. 1978; Camp 1986; Easley 1988; St. Jean *et al*. 1988).

4.6 Angles

Pregnant - postpartum cows

Table 4.02 depicts no significant change in the angle of pelvic girdle between different stages of gestation and postpartum. However, a slight and gradual increase in the pelvic girdle angle till 7 month of gestation (Figure 4.2) indicates an upward and backward movement of tuber coxae (Gautam and Nakao 2009). This could be attributed to a forward stretching of pubis and a concomitant backward movement of tuber coxae due to increased tension on the prepubic tendon by the developing fetus (Sisson and Grossman 1975). A decrease in the pelvic girdle angle after seven months of gestation may be attributed to a reduction in the traction on pubis and/or pelvic girdle because at this stage the fetus occupies the abdominal floor and begins an ascend into dorsal abdomen (Roberts 2004). The decrease in pelvic girdle angle

continued after calving and by first month postpartum the pelvic girdle angle simulated to the third month of gestation (Figure 4.2) thereby suggesting the return of pelvic girdle orientation to an early pregnancy stage.

Table 4.01 Comparison of age, parity and BCS in normal (n=30) and urovagina (n=15) cows

Serial No.	Age (years)		Parity		BCS	
	Normal	Urovagina	Normal	Urovagina	Normal	Urovagina
1	7.5	5.0	3	2	2.8	3.5
2	4.5	5.0	1	3	2.7	2.5
3	4.0	5.0	1	3	3.0	2.5
4	4.5	7.0	1	5	3.0	3.0
5	3.0	10.	1	4	2.7	3.5
6	6.0	16.0	2	8	3.0	3.5
7	4.0	15.0	1	6	3.0	3.8
8	6.0	6.0	2	2	2.8	3.8
9	5.0	10.0	2	8	2.7	3.0
10	6.0	4.0	1	1	2.7	3.5
11	6.0	7.0	2	4	3.0	2.2
12	4.0	7.0	1	2	2.5	3.0
13	8.0	7.0	3	2	2.8	3.0
14	14.0	5.0	6	3	3.0	3.5
15	4.0	8.0	1	4	2.8	3.0
16	5.0		2		3.0	
17	10.0		5		3.3	
18	2.5		1		3.0	
19	8.0		4		3.5	
20	6.0		2		2.8	
21	7.0		4		3.5	
22	8.0		4		3.0	
23	12.5		5		3.0	
24	4.0		1		2.7	
25	5.0		2		3.5	
26	3.0		1		3.2	
27	10.5		4		3.2	
28	12.0		5		3.0	
29	6.5		1		3.0	
30	9.0		5		3.3	
(mean ± S.E.M.)	**6.5 ± 0.5**	**7.8 ± 0.9**	**2.4 ± 0.3[a]**	**3.8 ± 0.5[b]**	**2.9 ± 0.04**	**3.1 ± 0.1**

- [a,b] Values in the same row differ at (P<0.01)

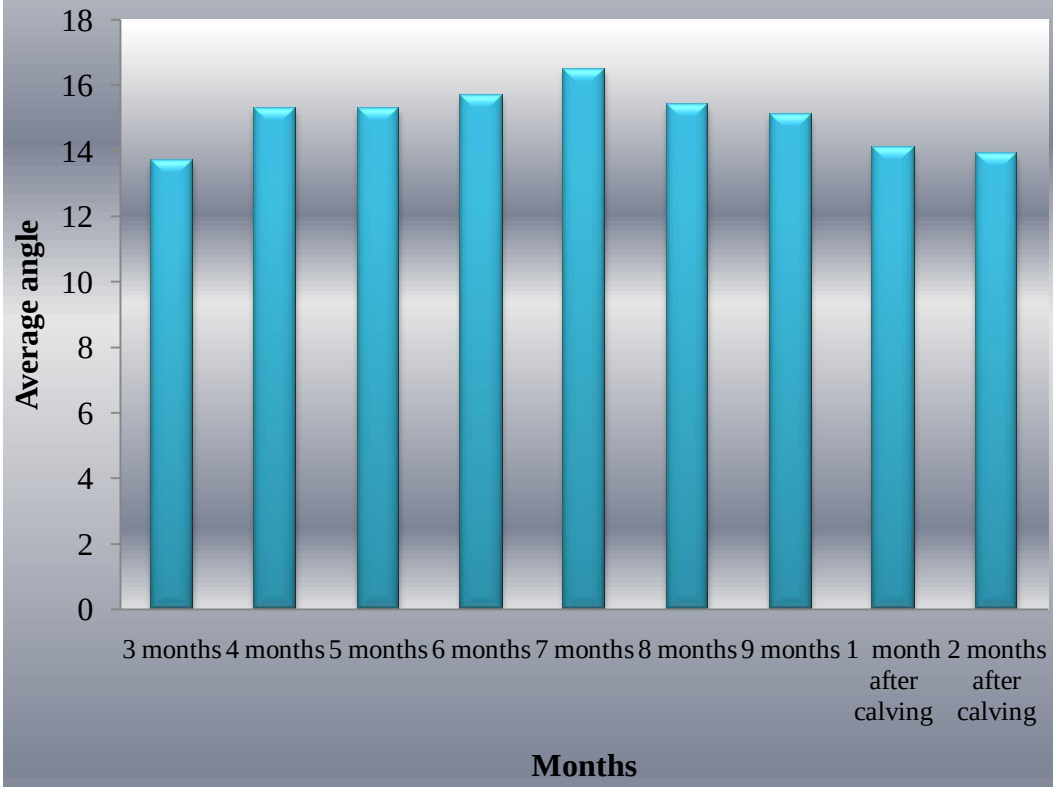
Table 4.02 Angles (degrees) of pelvic girdle at different gestation and postpartum stages in cows (n=31)

Serial No.	Month of gestation							Month after calving	
	3	4	5	6	7	8	9	1	2
1	-	17 (36)	17 (36)	18 (37)	19 (38)	19 (38)	18 (37)	18 (37)	13 (32)
2	-	19 (38)	15 (34)	15 (34)	15 (34)	17 (36)	17 (36)	13 (32)	11 (30)
3	-	24 (43)	15 (34)	16 (35)	23 (42)	21 (40)	19 (38)	17 (36)	15 (34)
4	-	19 (38)	17 (36)	16 (35)	24 (43)	18 (37)	20 (39)	14 (33)	14 (33)
5	-	14 (33)	13 (32)	12 (31)	15 (34)	15 (34)	16 (35)	12 (31)	13 (32)
6	-	14 (33)	16 (35)	17 (36)	20 (39)	14 (33)	15 (34)	14 (33)	12 (31)
7	-	19 (38)	23 (42)	25 (44)	27 (46)	19 (38)	20 (39)	16 (35)	17 (36)
8	-	20 (39)	17 (36)	16 (35)	25 (44)	18 (37)	19 (38)	20 (39)	15 (34)
9	-	13 (32)	16 (35)	18 (37)	15 (34)	15 (34)	14 (33)	14 (33)	14 (33)
10	-	17 (36)	20 (39)	19 (38)	16 (35)	15 (34)	23 (42)	15 (34)	15 (34)
11	-	21 (40)	19 (38)	18 (37)	19 (38)	20 (39)	20 (39)	16 (35)	17 (36)
12	-	-14 (5)	-13 (6)	-12 (7)	-18 (1)	-13 (6)	-16 (3)	-14 (5)	-12 (7)
13	-	22 (41)	21 (40)	20 (39)	26 (45)	18 (37)	19 (38)	20 (39)	18 (37)
14	26 (45)	20 (39)	21 (40)	20 (39)	17 (36)	18 (37)	13 (32)	16 (35)	17 (36)
15	18 (37)	16 (35)	16 (35)	21 (40)	16 (35)	17 (36)	14 (33)	15 (34)	15 (34)
16	15 (34)	18 (37)	18 (37)	19 (38)	17 (36)	16 (35)	14 (33)	14 (33)	15 (34)
17	17 (36)	20 (39)	21 (40)	17 (36)	17 (36)	18 (37)	13 (32)	15 (34)	20 (39)
18	18 (37)	13 (32)	13 (32)	20 (39)	19 (38)	17 (36)	16 (35)	15 (34)	17 (36)
19	15 (34)	13 (32)	12 (31)	19 (38)	17 (36)	16 (35)	15 (34)	14 (33)	16 (35)
20	-15 (4)	-13 (6)	-12 (7)	-17 (2)	-16 (3)	-16 (3)	-14 (5)	-14 (5)	-12 (7)
21	16(35)	20(39)	22 (41)	22 (41)	18 (37)	17 (36)	15 (34)	13 (32)	15 (34)
22	-	-	20 (39)	21 (40)	22 (41)	20 (39)	20 (39)	21 (40)	17 (36)
23	-	-	-	17 (36)	16 (35)	15 (34)	18 (37)	17 (36)	15 (34)
24	-	-	16 (35)	14 (33)	12 (31)	22 (41)	21 (40)	16 (35)	15 (34)
25	-	-	17 (36)	19 (38)	20 (39)	21 (40)	18 (37)	18 (37)	15 (34)
26	-	-	19 (38)	14 (33)	14 (33)	19 (38)	18 (37)	15 (34)	17 (36)
27	-	18 (37)	22 (41)	24 (43)	19 (38)	17 (36)	19 (38)	20 (39)	15 (34)
28	-	17 (36)	12 (31)	14 (33)	21 (40)	18 (37)	19 (38)	18 (37)	20 (39)
29	-	16 (35)	13 (32)	13 (32)	21 (40)	16 (35)	14 (33)	20 (39)	22 (41)
30	-	18 (37)	17 (36)	17 (36)	19 (38)	15 (34)	17 (36)	13 (32)	16 (35)
31	-	18 (37)	17 (36)	16 (35)	18 (37)	18 (37)	16 (35)	18 (37)	16 (35)
(mean ± S.E.M.)	**13.7 ± 4.2**	**15.3 ± 1.7**	**15.3 ± 1.4**	**15.7 ± 1.5**	**16.5 ± 1.7**	**15.4 ± 1.4**	**15.1 ± 1.5**	**14.1 ± 1.4**	**13.9 ± 1.3**

- Negative values indicate cranioventral tipping of the pelvis

- For data analysis, 19 was added to the entire data to convert -18, the lowest recorded negative value, to 1
- Numerics in the parenthesis are the transformed values
- (mean ± S.E.M.) represents the average of values not in parenthesis

Figure 4.2 Comparison of average (mean ± S.E.M.) pelvic girdle angles (degrees) at different gestation and post-partum stages in cows (n=31)



A change in gestation and postpartum stage did not yield a significant difference in the average angles (degrees) of vagina (Table 4.03). The average angle of vagina ranged from 7.3 ± 2.9 at second month postpartum to 9.8 ± 3.3 at seventh month of gestation. However, significanly higher angles of vulva were recorded during first month postpartum (17.3 ± 0.7) compared to fourth (21.6 ± 1.2) and

seventh (20.2 ± 0.8) month of gestation (Table 4.04). Inspite of significant difference, these values may not be of much practical / clinical relevance as there was a difference of approximately three degrees only.

Table 4.03 Angles (degrees) of vagina at different gestation and postpartum stage in cows (n=31)

Serial No.	Month of gestation							Month after calving	
	3	4	5	6	7	8	9	1	2
1	-	23 (54)	20 (51)	20 (51)	20 (51)	20 (51)	15 (46)	18 (49)	16 (47)
2	-	15 (46)	19 (50)	20 (51)	16 (47)	20 (51)	16 (47)	14 (45)	15 (46)
3	-	-12 (19)	-18 (13)	-20 (11)	-15 (16)	-18 (13)	-17 (14)	-18 (13)	-17 (14)
4	-	16 (47)	16 (47)	17 (48)	25 (56)	19 (50)	22 (53)	15 (46)	17 (48)
5	-	-24 (7)	-19 (12)	-20 (11)	-18 (13)	-26 (5)	-24 (7)	-24 (7)	-25 (6)
6	-	-16 (15)	-15 (16)	-14 (17)	-22 (9)	-16 (15)	-16 (15)	-17 (14)	-20 (11)
7	-	21 (52)	19 (50)	18 (49)	20 (51)	20 (51)	19 (50)	14 (45)	15 (46)
8	-	34 (65)	26 (57)	26 (57)	25 (56)	22 (53)	18 (49)	14 (45)	16 (47)
9	-	-16 (47)	-16 (15)	-18 (13)	-20 (11)	-18 (13)	-17 (14)	-14 (17)	-15 (16)
10	-	-14 (17)	-22 (9)	-23 (8)	-26 (5)	-16 (15)	-17 (14)	-18 (13)	-15 (16)
11	-	18 (49)	16 (47)	15 (46)	20 (51)	15 (46)	19 (50)	22 (53)	18 (49)
12	-	-29 (2)	-19 (12)	-20 (11)	-19 (12)	-27 (4)	-24 (7)	-24 (7)	-25 (6)
13	-	18 (49)	15 (46)	15 (46)	20 (51)	18 (49)	18 (49)	14 (45)	17 (48)
14	18 (49)	18 (49)	17 (48)	20 (51)	18 (49)	19 (50)	14 (45)	16 (47)	18 (49)
15	15 (46)	17 (48)	19 (50)	21 (52)	29 (60)	16 (47)	13 (44)	20 (51)	16 (47)
16	14 (45)	27 (58)	24 (55)	17 (48)	20 (51)	18 (49)	12 (43)	20 (51)	17 (48)
17	18 (49)	24 (55)	24 (55)	20 (51)	18 (49)	18 (49)	12 (43)	19 (50)	16 (47)
18	17 (48)	17 (48)	18 (49)	20 (51)	18 (49)	17 (48)	15 (46)	17 (48)	16 (47)
19	14 (17)	-18 (13)	-20 (11)	-16 (15)	-18 (13)	-16 (15)	-13 (18)	-17 (14)	-17 (14)
20	-29 (2)	-24 (7)	-22 (9)	-20 (11)	-20 (11)	-23 (8)	-16 (15)	-30 (1)	-23 (8)
21	20 (51)	23 (54)	24 (55)	21 (52)	22 (53)	18 (49)	16 (47)	14 (45)	16 (47)
22	-	-	24 (55)	24 (55)	25 (56)	21 (52)	20 (51)	23 (54)	14 (45)
23	-	-	-	18 (49)	21 (52)	22 (53)	18 (49)	19 (50)	14 (45)
24	-	-	15 (46)	14 (45)	13 (44)	25 (56)	23 (54)	18 (49)	18 (49)
25	-	-	30 (61)	25 (56)	23 (54)	20 (51)	22 (53)	25 (56)	14 (45)
26	-	-	22 (53)	17 (48)	15 (46)	18 (49)	21 (52)	14 (45)	16 (47)
27	-	20 (51)	19 (50)	18 (49)	24 (55)	19 (50)	18 (49)	15 (46)	21 (52)
28		24 (55)	20 (51)	20 (51)	20 (51)	20 (51)	13 (44)	16 (47)	18 (49)
29		18 (49)	14 (45)	12 (43)	19 (50)	13 (44)	12 (43)	18 (49)	20 (51)
30		21 (52)	18 (49)	16 (47)	16 (47)	17 (48)	15 (46)	14 (45)	18 (49)
31		19 (50)	15 (46)	13 (44)	17 (48)	19 (50)	16 (47)	19 (50)	18 (49)
(mean ± S.E.M.)	**7.3 ± 6.4**	**9.6 ± 3.6**	**9.4 ± 3.2**	**8.9 ± 3.0**	**9.8 ± 3.3**	**8.8 ± 3.1**	**7.8 ± 2.8**	**7.6 ± 3.0**	**7.3 ± 2.9**

- Negative values indicate cranioventral displacement of vagina.

- For data analysis, 31 was added to the entire data to convert -30, the lowest negative value, to 1.
- Numerics in the parenthesis are the transformed values
- (mean ± S.E.M.) represents the average of values not in parenthesis

Table 4.04 Angles (degrees) of vulva at different gestation and postpartum stages in cows (n=31)

Serial No.	Month of gestation							Month after calving	
	3	4	5	6	7	8	9	1	2
1	-	14	14	15	14	12	18	12	15
2	-	26	22	22	22	16	18	14	18
3	-	27	27	29	18	20	12	19	19
4	-	18	17	17	22	15	18	14	11
5	-	23	20	20	20	22	24	13	24
6	-	33	25	23	25	22	24	21	28
7	-	25	30	32	30	42	40	19	18
8	-	25	15	15	16	12	21	15	21
9	-	17	21	23	17	14	13	12	16
10	-	16	16	15	13	20	16	17	15
11	-	19	23	24	26	21	34	21	21
12	-	21	28	28	29	30	32	30	34
13	-	16	19	20	15	17	15	13	15
14	17	11	11	11	15	16	15	13	16
15	18	18	17	16	20	15	12	14	20
16	23	14	15	17	16	17	18	17	18
17	20	15	13	16	18	17	14	19	15
18	25	20	18	24	24	16	25	27	25
19	20	20	19	21	27	25	16	18	19
20	25	35	34	22	25	20	15	27	39
21	17	18	19	14	19	15	14	16	16
22	-	-	15	14	13	15	14	14	11
23	-	-	-	16	20	20	26	16	11
24	-	-	17	24	25	19	20	14	17
25	-	-	11	16	18	15	15	15	17
26	-	-	15	19	20	15	17	19	16
27	-	32	20	20	20	21	20	19	20
28	-	30	26	24	23	21	27	17	15
29	-	20	16	15	17	24	20	15	17
30	-	19	16	15	15	23	14	17	16
31	-	30	26	22	25	28	22	20	22
(mean ± S.E.M.)	**20.6 ± 1.7**	**21.6 ± 1.2[a]**	**19.5 ± 1.0**	**19.6 ± 0.8**	**20.2 ± 0.8[a]**	**19.5 ± 1.0**	**19.6 ± 1.2**	**17.3 ± 0.7[b]**	**18.8 ± 1.0**

- [a,b] Values differ at ($P<0.05$)

Table 4.05 shows Pearson correlation coefficient between angles of pelvic girdle, vagina and vulva. A significant positive correlation between pelvic girdle and vagina indicated an increase in angle of vagina with an increase in angle of pelvic girdle. For example with an upward shift in tuber coxae the vagina also shifts in a manner so that the fornix vagina is at a higher level than the posterior vagina. Change in angulations of vagina with a change in that of pelvic girdle may be due to the relation of lateral part of vagina to pelvic girdle (Sisson and Grossman 1975). However, there was a significant and negative correlation between angles of pelvic girdle and vulva i.e. as the angle of pelvic girdle increases the angle of vulva decreases. The upward and backward movement of tuber coxae causes posterior movement of dorsal commissure of vulva in a way that both move in the same direction. However, the negative association may be due
to a difference in the orientation of axis of pelvic girdle (horizontal axis; Plate-3.1, 3.2) and vulva (vertical axis; Plate–3.5). The movement of vulva and pelvic girdle in the same direction may be due to lateral attachment of vulva to semimembranosus muscle and sacro-sciatic ligament, which together form the lateral boundary of pelvic outlet (Sisson and Grossman 1975).

Table 4.05 Pearson correlation coefficient between angles (degrees) of pelvic girdle, vulva and vagina recorded at different gestation and postpartum stages

	Pelvic girdle	Vagina	Vulva
Pelvic girdle	1.00	0.568 <0.01	-0.358 <0.01
Vagina		1.00	
Vulva		-0.298 <0.01	1.00

- Negative sign indicates reciprocal correlation.

The angles of pelvic girdle at different days of estrus in normal and urovagina cows are presented in Table 4.06. The pelvic girdle was craniodorsal at Day 0 in all the normal cows which, however, became cranioventral at Day 14 in two normal cows. Compared to normal cows, 7 out of 15 urovagina cows at Day 0 had a cranioventral pelvic girdle, whereas cranioventral pelvic girdle was evident in 14 out of 15 cows at Day 14 (Figure 4.3). These findings are supplemented by no significant difference in pelvic girdle angles between Day 0 and Day 14 in the normal cows (15.7 ± 0.5 verses 14.0±1.3)

4.7 Angles

Non-Pregnant cows

Table 4.06 Angles (degrees) of pelvic girdle at different stages of estrous cycle in the normal (n=30) and the urovagina (n=15) cows

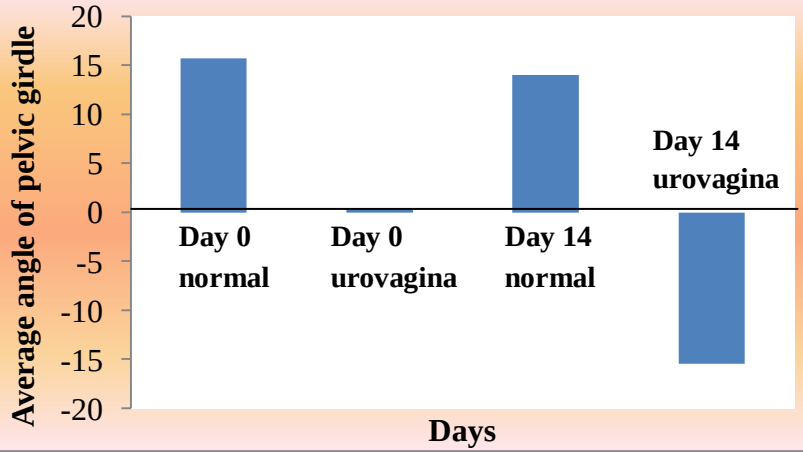
Serial No.	Stage of estrous cycle			
	Day 0		Day 14	
	Normal	Urovagina	Normal	Urovagina
1	19 (55)	20 (56)	15 (51)	-11 (25)
2	18 (54)	19 (55)	16 (52)	12 (48)
3	18 (54)	10 (46)	15 (51)	-10 (26)
4	18 (54)	23 (59)	15 (51)	-12 (24)
5	13 (49)	13 (49)	12 (48)	-11 (25)
6	16 (52)	-14 (22)	21 (57)	-15 (21)
7	18 (54)	-32 (4)	13 (49)	-30 (6)
8	14 (50)	-25 (11)	-13 (23)	-17 (19)
9	15 (51)	12 (48)	12 (48)	-35 (1)
10	16 (52)	32 (68)	15 (51)	-12 (24)
11	15 (51)	-16 (20)	15 (51)	-18 (18)
12	14 (50)	-30 (6)	16 (52)	-28 (8)
13	20 (56)	-10 (26)	18 (54)	-15 (21)
14	14 (50)	-14 (22)	15 (51)	-10 (26)
15	11 (47)	17 (53)	-12 (24)	-19 (17)
16	16 (52)		15 (51)	
17	18 (54)		15 (51)	
18	14 (50)		20 (56)	
19	15 (51)		17 (53)	
20	19 (55)		16 (52)	
21	12 (48)		10 (46)	
22	16 (52)		20 (56)	
23	18 (54)		14 (50)	
24	20 (56)		18 (54)	
25	15 (51)		17 (53)	
26	18 (54)		20 (56)	
27	13 (49)		18 (54)	
28	13 (49)		15 (51)	
29	11 (47)		15 (51)	
30	15 (51)		17 (53)	
(mean ±S.E.M.)	**15.7 ± 0.5[c]**	**0.3 ± 5.4[a]**	**14.0 ± 1.3[d]**	**-15.4 ± 2.8[b]**

- Negative values indicate cranioventral pelvis.
- For data analysis, 36 was added to the entire data to convert -35, the lowest recorded value, to 1.
- Numerics in the parenthesis are the transformed values
- (mean ± S.E.M.) represents the average of values not in parenthesis
- [a,b; a,c and b,d] Values with different superscripts in same row differ at ($P<0.01$) and ($P<0.0001$), respectively.

this, however, was significant in the urovagina cows (0.3 ± 5.4 verses -15.4 ± 2.8). Hence, more number of urovagina cows than the normal cows with cranioventral pelvic girdle at Day 0 and Day 14 attributed to significantly lower corresponding angles in the former (Table 4.06).

Cranioventral tipping of pelvis has been quoted as a potential reason of urovagina in cows (Hudson 1986; Cox 1987; St. Jean *et al*. 1988; Prado *et al.* 2007) and mares (Zemzanis 1970; Lieux 1972; Monin 1973; Vaughn 1974; Thornbury 1975 Brown *et al.* 1978). Cranioventral pelvis raises the external urethral orifice higher than the adjacent vaginal floor during estrus thereby gravitating the urine into vaginal fornix (Cox 1987; St. Jean *et al*. 1988). A similar reason could be implicated as a cause of urovagina in the cows of current study as much higher number of the urovagina cows had cranioventral pelvic girdle.

Figure 4.3 Average pelvic angle at Day 0 and Day 14 in the normal (n=30) and the urovagina (n=15) cows



The angles of vagina at different days of estrous cycle in the normal and the urovagina cows at Day 0 and Day 14 are presented in (Table 4.07). As evident 53.3% (16/30) of the normal cows at Day 0 had cranioventral vagina, compared to 40.0% (12/30) at Day 14. Almost similar proportion of urovagina cows had cranioventral vagina at Day 0 and Day 14, the values being 40.0% (6/15) and 46.6% (7/15), respectively. The average angles of vagina between Day 0 and Day 14 were similar in normal and urovagina cows and did not differ between the two groups of cows. Our findings on vaginal angles, as a cause of urovagina, do not support previous reports indicating cranioventral displacement of vagina in the urovagina cows (St. Jean *et al*. 1988; Gautum

Table 4.07 Angles (degrees) of vagina at different stages of estrous cycle in the normal (n=30) and the urovagina (n=15) cows

Serial No.	**Stage of estrous cycle**			
	Day 0		**Day 14**	
	Normal	Urovagina	Normal	Urovagina
1	-19 (24)	32 (75)	18 (61)	30 (73)
2	-17 (26)	12 (55)	17 (60)	9 (52)
3	16 (59)	43 (86)	16 (59)	31 (74)
4	19 (62)	21 (64)	14 (57)	34 (77)
5	18 (61)	33 (76)	16 (59)	19 (62)
6	-18 (25)	19 (62)	-15 (28)	25 (68)
7	20 (63)	-42 (1)	17 (60)	-30 (13)
8	13 (56)	28 (71)	-15 (28)	20 (63)
9	14 (57)	22 (65)	-14 (29)	21 (64)
10	14 (57)	23 (66)	12 (55)	-10 (33)
11	13 (56)	-21 (22)	-18 (25)	-24 (19)
12	13 (56)	-23 (20)	14 (57)	-25 (18)
13	-17 (26)	-15 (28)	-15 (28)	-18 (25)
14	-18 (25)	-30 (13)	-20 (23)	-32 (11)
15	-20 (23)	-32 (11)	-15 (28)	-35 (8)
16	-15 (28)		-16 (27)	
17	-16 (27)		-17 (26)	
18	15 (58)		20 (63)	
19	14 (57)		16 (59)	
20	20 (63)		18 (61)	
21	-17 (26)		17 (60)	
22	12 (55)		17 (60)	
23	-16 (27)		19 (62)	
24	-16 (27)		-14 (29)	
25	14 (57)		16 (59)	
26	-20 (23)		-16 (27)	
27	-16 (27)		19 (62)	
28	-20 (23)		-23 (20)	
29	-20 (23)		17 (60)	
30	-20 (23)		17 (60)	
(mean ±	**-2.3 ± 3.1**	**4.7 ± 7.3**	**3.4 ± 3.0**	**1.0 ± 6.7**

S.E.M.)				

- Negative values indicate cranioventral of vagina
- For data analysis, 43 was added to the entire data to convert -42, the lowest recorded value, to 1.
- Numerics in the parenthesis are the transformed values
- (mean ± S.E.M.) represents the average of values not in parenthesis

and Nakao 2009) and mares (Easley 1988). However, a species specific variation in the orientation of vagina cannot be precluded. All the previous studies on urovagina are either in Holstein (Gilbert et al. 1989; Farhoodi et al. 2000; Gautum and Nakao 2009; Gonzalez-Martin 2008) or Charolais (Noakes *et al*. 2009) cows compared to Jersey crossbred cows in the present study. Holstein and Charolais are reported to be more vulnerable to urovagina (Noakes *et al.* 2009).

The angles of vulva at different days of estrous cycle are presented in Table 4.08. There was no difference in the average vulvar angle both within and between the Day 0 and Day 14 values in the normal and the urovagina cows. All the normal and urovagina cows had positive values of vulva angles, which is suggestive that the vulva orientation is more towards the vertical axis, also designated as vertical vulva (Gautum and Nakao 2009).

St. Jean *et al*. (1988) and Gautum and Nakao (2009) reported horizontal tipping of vulva manifested by anterior movement of dorsal vulvar commissure as a characteristic of urovagina in 71.0% and 77.8% cows, respectively. There is a close association between the anal sphincter, constrictor vulvae muscle and constrictor vestibuli muscle. Damage to anyone of these may lead to cranioventral vagina and vestibule with a sunken anus (Easley 1988; St. Jean *et al*. 1988) and horizontal tipping of vulva (St. Jean *et al*. 1988) in urovagina cows. However, lack of any difference in the vaginal angles (Table 4.07) and absence of a sunken anus justifies a normalcy in vulvar angles and also rules out damage to the constrictor vestibuli muscle in the urovagina cows of present study. Hence, cranioventral pelvic girdle and its probable association with abnormal location of external urethral orifice (higher than vaginal

floor) may be a more plausible reason of urovagina in the Jersey crossbred cows of the current study.

There was a non-significant correlation between the angles of pelvic girdle, vagina and vulva in the normal cows. However, there was a significant correlation between angles of pelvic girdle-vagina and vagina-vulva in the urovagina cows (Table 4.09). A significant and positive correlation between pelvic girdle and vaginal angle in the urovagina cows of present study is suggestive of cranioventral movement of vagina with cranioventral tipping of pelvis and vice-versa. Both cranioventral vagina (St. Jean *et al*. 1988) and cranioventral pelvis (Hudson 1986) have been recorded in the urovagina cows. Relationship between the vagina and vulva angles was also significant in the urovagina cows and was suggestive of a trend in the movement of vagina and

Table 4.08 Angles (degrees) of vulva at different stages of estrous cycle in the normal (n=30) and the urovagina (n=15) cows

Serial No.	Stage of estrous cycle			
	Day 0		Day 14	
	Normal	Urovagina	Normal	Urovagina
1	37	23	23	20
2	17	13	17	23
3	15	30	13	19
4	25	33	26	21
5	14	48	16	31
6	17	9	17	11
7	23	82	18	82
8	16	15	16	13
9	22	14	21	20
10	26	17	29	24
11	39	28	30	25
12	17	30	14	28
13	16	30	16	18
14	11	28	20	30
15	38	30	45	34
16	29		19	
17	12		16	
18	38		36	
19	18		16	
20	26		28	
21	43		40	
22	20		21	
23	14		15	
24	15		15	

25	18		16	
26	19		19	
27	27		24	
28	37		45	
29	16		20	
30	36		25	
(mean ± S.E.M)	**23.3 ± 1.7**	**28.6 ± 4.6**	**22.5 ± 1.6**	**26.6 ± 4.2**

Table 4 .09 Pearson correlation coefficient between angles (degrees) of pelvic girdle, vulva and vagina (combined for Day 0 and Day 14) in the urovagina (n=15) cows

	Pelvic girdle	**Vagina**	**Vulva**
Pelvic girdle	1	0.424 P<0.05	-0.256 Non significant
Vagina		1	-0.483 P<0.01
Vulva			1

vulva in same direction. This re-affirms to some previous studies in which cranial movement of vagina (Hudson 1986; Gonzalez-Martin 2008) and cranial movement of vulva (St. Jean *et al*. 1988: Ewoldt 2009; Gautum and Nakao 2009) are associated. The negative association between vagina and vulva angles indicate a downward displacement of vagina (decrease in angle) with a cranial displacement of dorsal commisure of vulva (increase in angle). The negative association was, however, due to horizontal axis of vagina and vertical axis of vulva.

4.8 Regression equation

Regression equation was framed by using statistically significant variables (pelvic girdle angle, parity and estrous duration) in the urovagina cows to determine the probability of urovagina in other cows. The equation was as follows:

$$Y = - 0.035 - 0.006x + 0.008z + 0.374b$$

Y = Probability of urovagina

x = Value of pelvic girdle angle

z = Parity

b = Estrous duration.

4.9 Histopathology

4.9.1 Vagina

The histological findings of vaginal musculature revealed compact smooth muscle fibres with spindle shaped nuclei and uniform cytoplasm in the normal cows (Plate 4.1). However, the vaginal biopsy revealed thin smooth muscle fibres with oval shaped nuclei in the urovagina cows. Also, the muscle fibres in the later cows were more eosinophilic suggesting a degenerative change as also supported by the vacuolar degeneration of muscle fibres at some places (Hulland 1985) (Plate 4.2).

Hence, abnormal pathological findings of vaginal smooth muscles around external urethral orifice in the affected cows hint abnormal urine expulsion in the urovagina cows.

4.9.2 Uterus

Uterine tissue of normal cows showed a marked increase in endometrial activity. Endometrium appeared loose and there was a marked congestion of blood vessels. There was a normal neutrophil and mononuclear cellular infiltration. Endometrial glands were free of cellular debris and inflammatory cells (Plate 4.3). These changes corroborate to a healthy uterine endometrium of estrus phase as reported earlier (Lohuis *et al*. 1992).

However, in urovagina cows the sections of uterus revealed infiltration of superficial layer with a large number of neutrophils, macrophages and occasional lymphocytes and fibroblasts. The endometrial glands also showed hyperplastic changes and cellular debris in their lumen (Plate 4.4). Larger number of blood vessels were also opened up and deeper tissues were heavily infiltrated with fibrous tissues. These findings confirm the presence of chronic endometritis (Shukla 1988, Sawamukai *et al*. 1994) in the urovagina cows of present study.

4.10 Blood serum calcium concentration

There was no difference in the average calcium concentration between the normal and the urovagina cows (Table 4.10). Hence, calcium deficiency in relation to abnormal functioning of muscle (Bailey 2004) involved in urination in the urovagina cows of present study, is ruled out.

4.11 Surgical correction

No signs of discomfort were observed in any cow during surgical procedure. Haemorrhage was rare. The time required for surgery ranged from 7-35 min. Four cows received no antimicrobial treatment post surgery. One cow showing signs of vaginitis on the second day after surgery received Dicrysticin-S (Sarabhai Zydus Animal Health Limited, each vial contains, Streptomicin 2.5g, Procaine Penicillin 15,00,000 units and Penicillin G Sodium 500,000 units) and Melonex® Plus (Intas Pharmaceuticals Limited, each ml contains, Meloxicam 5mg and Paracetamol 150 mg) for seven days. Re-examination of the operated cows seven days after surgery indicated the retention of suture in three out of five (60%) cows with maintenance of one finger constriction (Plate 4.6). All the three cows retaining the suture exhibited a complete absence of urine in the genital discharge at subsequent estrous (Table 4.11). The results of surgical success in present study are lower than 89.5% (17/19) recorded by Gonzalez-Martin *et al*. (2008).

Table 4.10 Serum calcium concentration (g/dl) in the normal (n=10) and the urovagina (n=11) cows

Serial No.	Serum calcium concentration	
	Normal	Urovagina
1	9.65	7.80
2	10.03	10.74
3	10.55	9.59
4	9.20	10.49
5	8.66	9.17
6	9.04	8.09
7	8.73	10.44
8	8.76	10.26
9	7.71	10.44
10	8.16	9.91
11	NR	14.19
(mean ± S.E.M)	9.0 ± 0.2	10.1 ± 0.5

NR- Not recorded

Table 4.11 Change in the volume (ml) of urovaginal contents before and after surgical treatment in five urovagina cows

Serial. No.	Before treatment	After treatment
1	48	45
2	80	40
3	100	Nil
4	145	Nil
5	200	Nil

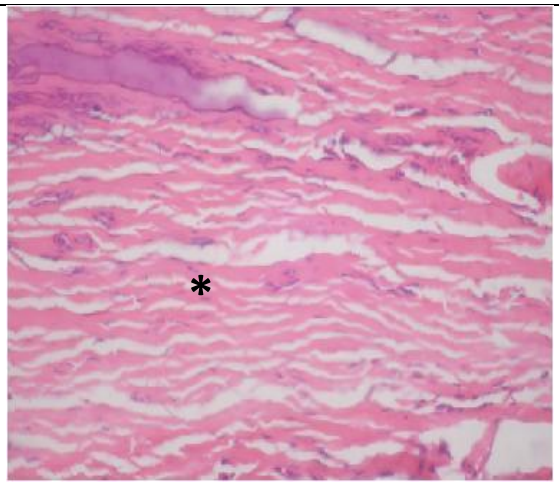
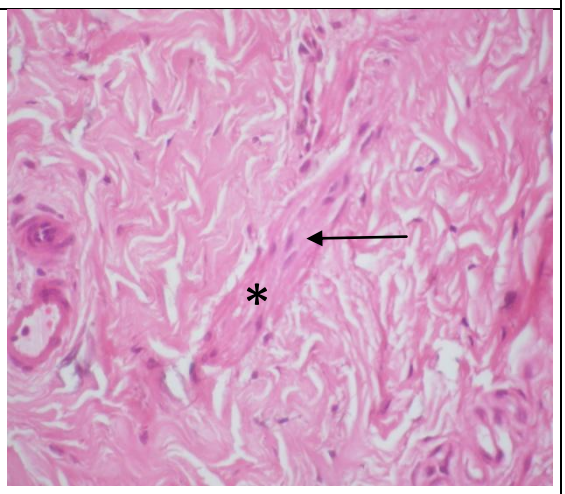
Plate 4.1 Micrograph showing spindle shaped nuclei and uniform cytoplasm (astrix) (H& E 132x) in the vaginal section of a normal cows.	**Plate 4.2 Micrograph showing oval nuclei (arrow) and vacuolated cytoplasm (astrix) (H& E 132x) in the vaginal section of an urovagina cows.**
	

Plate 4.3 Micrograph showing active endometrial gland	**Plate 4.4 Micrograph showing cellular debris in the**

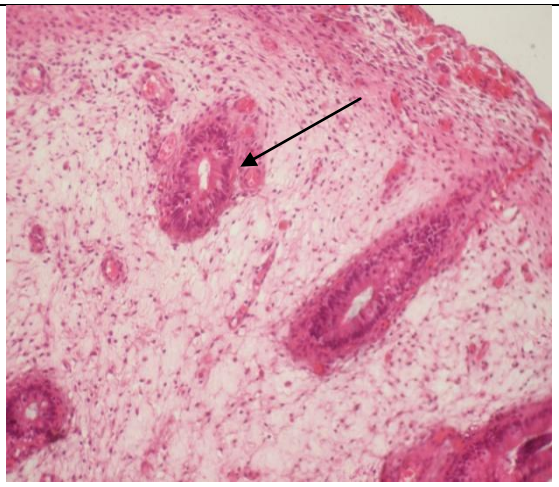
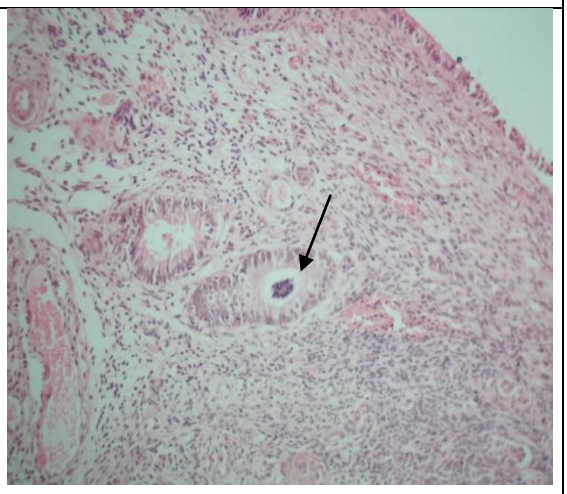
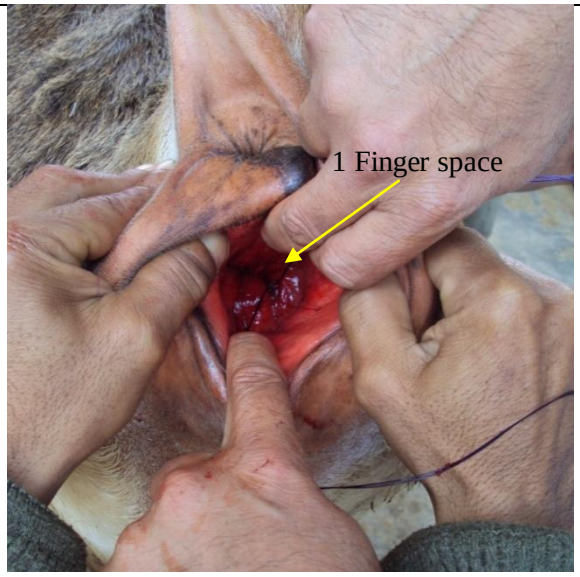
(arrow) (H& 66x) in uterine section of the normal cows	**endometrial gland (arrow) (H& E66x) in uterine section of the urovagina cows**
	

Plate 4.5 Vestibulovaginal junction before surgery	**Plate 4.6 Vestibulovaginal junction after surgery**
	

SUMMARY AND CONCLUSIONS

Chapter 5

SUMMARY AND CONCLUSIONS

Optimum cattle reproduction is a pre-requisite for a successful dairy enterprise. Urovagina or pooling of urine in vaginal fornix hampers the spermatozoa and embryonic survival and is therefore a serious impediment for the fertility. The present study intended to test the hypothesis of pregnancy associated changes in the angulations of pelvic girdle, vagina and vulva and presence of urovagina. The second part of the study was planned to have an insight into the possible etiology and efficacy of surgical treatment in the urovagina cows. The available studies on urovagina are either in mare or in Holstein and Charolais cattle raised abroad. There are no studies on urovagina in the Indian cows.

The present study was designed in the Jersey crossbred cows with the following objectives:

i) To investigate the cows under different stages of reproduction for detection of urovagina.

ii) To treat urovagina in cows.

The study was carried out in two parts. In the first part, 31 cows were examined at monthly intervals from third month of gestation till second month postpartum. At each examination, the cows were subjected to vaginoscopy for detection of urovagina and measurement of angles of pelvic girdle, vagina and vulva, respectively.

The angles of pelvic girdle and vagina did not differ significantly between different pregnancy and postpartum stages. However, the vulvar angles of 21.6 ± 1.2 and 20.2 ± 0.8 at fourth and seventh month of pregnancy, respectively, were higher ($P<0.05$) than 17.3 ± 0.7 recorded at first month after calving. This difference of approximately three degrees was inferred to be too small and of any practical significance. The angles of pelvic girdle, vagina and vulva were significantly correlated among each other.

In the second part of study, cyclic and non-pregnant cows (30 normal and 15 urovagina affected) were examined once at Day 0 (estrus) and Day 14 (14 days after end of estrus) for different parameters. At Day 0, vaginoscopy was undertaken to confirm the presence and severity of urovagina. The vaginal contents were collected

and evaluated for colour, spinbarkeit, volume, odour and pH. The function of constrictor vestibule muscle was examined by inserting the little finger and recording the time taken to restore its normal closure after removal of finger. In addition, the past reproductive problems and current reproductive status including estrous duration and breeding history were recorded. Age parity, body condition score, biopsies of vaginal smooth muscles around urethra and uterine endometrium and blood sample for serum calcium estimation were undertaken at Day 0. The angles of pelvic girdle, vagina and vulva were measured both at Day 0 and Day 14.

Surgery, comprising of vestibulovaginal cerclage, was undertaken in five urovagina cows.

Vaginoscopy confirmed the pooling of urine in the vaginal fornix of urovagina cows and depending on the extent of dipping of cervical external-os, one (6.6%), eight (53.3%) and six (40.0%) cows were detected with mild, moderate and severe urovagina, respectively. Vaginal contents were clear, stringy, 17.2 ± 0.3 ml in volume and had a pH of 7.01 ± 0.01 in normal cows compared to yellowish, watery, significantly larger volume of 107.6 ± 10.5 ml and a much higher pH of 8.3 ± 0.01 in the urovagina cows. The external urethral orifice closed in <1 sec in both the normal and the urovagina cows, indicating a normal functioning of the constrictor vestibule muscle. The normal and urovagina cows did not reveal any evidence of peripartum problems in previous gestations. However, the current performance of all the urovagina cows indicated them to be repeat breeders and presence of prolonged estrus was a consistent feature. The average estrous duration was 2.8 ± 0.7 d and 1.1 ± 0.05 d ($P<0.0001$) in normal and urovagina cows, respectively. The urovagina cows were higher in parity than the normal cows (3.8 ± 0.5 versus 2.4 ± 0.3; $P<0.01$). The pelvic girdle was inclined more towards cranioventral side in urovagina compared to normal cows at Day 0 (0.3 ± 5.4 versus 15.7 ± 0.5; $P<0.01$) with a further increase in the cranioventral tipping at Day 14 in the urovagina cows without a much change in normal cows (- 15.8 ± 2.8 versus 14.0 ± 1.3; $P<0.0001$) compared to Day 0 values. The vagina and vulva angulations did not reveal any difference at Day 0 and Day 14 between the normal and the urovagina cows. The correlation matrix revealed a significant association ($P<0.05$ atleast) between pelvic girdle-vagina and vulva-vagina, respectively, in the urovagina cows.

Histological examinations of vagina indicated compact smooth muscles fibers with uniform cytoplasm and cylindrical nuclei suggesting normal muscles in the

normal cows. However, the muscles were thin with oval shaped nuclei alongwith vacuolated cytoplasm indicating vacuolar degeneration in the urovagina cows. Histological examination of the uterine endometrium exhibited an increased endometrial activity with moderate neutrophilic infiltration reflecting estrus. However, there was heavy infiltration of neutrophils, macrophages and occasional lymphocytes in the superficial layer of endometrium along with debris and inflammatory cells in the endometrial gland in the urovagina cows; the changes *per se* indicated endometritis.

Serum calcium concentrations were similar in urovagina and normal cows (10.1 ± 0.5 versus 9.0 ± 0.2 g/dl

The vestibulovaginal cerclage technique was effective in three out of the five cows (60% efficacy). The cows with clinical recoveries did not exhibit any urine in the genital discharge at the estrus subsequent to surgery.

The conclusions drawn from this study are:

In Jersey crossbred cows –

1. Different pregnancy and postpartum stages in 31 cows neither revealed urovagina nor any appreciable change in the angulations of pelvic girdle, vagina and vulva.
2. Moderate degree of urovagina was most common in the 15 urovagina affected cows.
3. Cows with higher parity, cranioventral tipping of pelvis and prolonged estrus were the most likely candidates to be affected with urovagina.
4. The histopathology of vaginal smooth muscle indicated a vacuolar degeneration in the urovagina cows, which could be a probable reason of urine pooling in these cows. The histopathology of uterine endometrium indicated endometritis in all the urovagina cows.
5. Vestibulovaginal cerclage technique resolved urovagina in 60% cows.

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