

# Laparoscopic diagnosis of gross reproductive abnormalities in free-ranging female rhesus macaques (*Macaca mulatta*)

Vijay Kumar,<sup>1</sup> Anshu Raj<sup>2</sup>

<sup>1</sup>Dhauladhar Nature Park, Gopalpur, Distt-Kangra, Himachal Pradesh;

<sup>2</sup>Veterinary Hospital, Deol, Baijnath, Distt-Kangra, Himachal Pradesh, India

## Abstract

A study was conducted between July 2010 to June 2011 in the Monkey Sterilization Centre, Gopalpur, Himachal Pradesh, India to assess the prevalence of reproductive disorders by laparoscopic examination of the genitalia of female rhesus macaques. The animals were captured from different locations in the state using a cage trapping method. A total of 720 female rhesus macaques underwent laparoscopic examination of their reproductive tracts. A total of 63 cases were found to have reproductive abnormalities. Out of these 63 cases, the most common abnormalities reported were ovarian cyst 3.05% (n=22), ovarian tumor 1.66% (n=12), uterine tumor 1.25% (n=9), uterine edema 0.97% (n=7), uterine rupture 0.83% (n=6), ectopic pregnancy 0.69% (n=5), unicornis or acornis 0.28% (n=2). The highest number of cases of uterine abnormalities was recorded in the 12-16 year old age group (33.33%), followed by 8-12 year olds (25.39%), 16-20 year olds (22.22%), then 4-8 year olds (15.87%) and 0-4 year olds (3.17%). Of the 63 animals that showed genital abnormalities, only 52 were found to be pregnant during the breeding season from November to March in the 5-20 year old age group, while 11 animals were not pregnant; a pregnancy rate of 82.53% (52 of 63) of the female rhesus macaques with reproductive abnormalities.

## Introduction

There have been significant advances in the diagnosis and treatment of reproductive disorders. Diagnostic laparoscopy is currently being utilized on a large scale in humans as well as in animals throughout the world. This is a safe and fast technique that can be performed under general or occasionally local anesthesia. Laparoscopy has been extensively used for the diagnosis and treatment of vari-

ous reproductive disorders in human females,<sup>1-3</sup> while in non-human primates, research has also been carried out on the reproductive physiology of the female rhesus macaques throughout the world.<sup>4-8</sup> However, there are still few data on laparoscopic diagnosis of the genital abnormalities in the female rhesus macaques and only a few cases have been reported. The reproductive abnormalities in captive and free-ranging rhesus macaques observed by laparoscopy have received little attention.<sup>9</sup> Rhesus macaques, the old world non-human primates, are considered a human model for various types of reproductive research, such as hormonal secretions, menstrual cycle and morphological characteristics of the reproductive systems, since these are similar to human females.<sup>10</sup> Primary or acquired reproductive abnormalities result from developmental defects, or genetic and environmental factors, or a combination of intrinsic and extrinsic factors.

In this study, rhesus macaques were captured by cage trapping methods on a large scale by the Wild Life Unit in Himachal Pradesh for the purpose of population control of rhesus macaques. Trapping has allowed data to be collected on reproductive abnormalities in female rhesus macaques. The aim of this study was to determine the reproductive abnormalities in female rhesus macaques by laparoscopic examination of female genitalia.

## Materials and Methods

The study was conducted in the Shivalik Hills region of the state with altitude ranging from 350 to 1500 meters. The animals were captured in different locations by a cage trapping method between July 2010 and June 2011. The baits used to attract monkeys to enter the cage were selected on the basis of the natural feed available to monkeys in those areas. After capture, the monkeys were transported to the Monkey Sterilization Centre. The study was carried out on 720 female rhesus macaques. Animals were rested for 1-2 days before laparoscopy. The animals with distended abdomen were identified to be in late stage of pregnancy by visual examination in individual cages and were kept separately. Animal age was determined on the basis of teeth eruption and physical status.

Animals were anesthetized after 15 h fasting with a combination of Xylazil® (2 mg/kg body weight) Ketamil® (10 mg/kg body weight) (Troy Laboratories Pty Ltd., Australia), mixed in a 2 mL disposal syringe with a 22 gauge needle. Animals in early and mid-stage of pregnancy were given combination dose of xylazine and ketamine while animals in late stage of pregnancy were injected only with ketamine at

Correspondence: Vijay Kumar, Veterinary Officer, Vill-Laka, P.O - Sarkaghat, Distt- Mandi, PIN 175024, India. Tel. +91.9459.133330. E-mail : drvijay0220@gmail.com

Key words: laparoscopic diagnosis, reproductive abnormalities, rhesus macaques (*Macaca mulatta*).

Acknowledgements: the authors are thankful to D.F.O Wild Life - Hamirpur Sh. Satish Gupta, ACF - Smt Sangeeta as well as all the team of monkey sterilization, Gopalpur, for their assistance throughout the work.

Conflict of interests: the authors report no conflict of interests.

Received for publication: 18 October 2011.

Revision received: 24 April 2012.

Accepted for publication: 10 September 2012.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright V. Kumar and A. Raj, 2012  
Licensee PAGEPress srl, Italy  
Veterinary Science Development 2012; 2:e13  
doi:10.4081/vsd.2012.e13

15 mg/kg body weight. All the animals were anesthetized in a standard size squeeze cage. All the animals were divided into five age groups as shown in Table 1.

Animals were prepared for surgery after proper shaving and scrubbing. The animal was then placed in Trendelenburg's position in dorsal recumbency (head lower than body) on a tilted table in the operating theater.

Standard laparoscopic procedure was adopted for all animals. A pre-umbilical incision was made with a scalpel and a Veress needle (2 mm × 80 mm) was inserted through this port to achieve pneumoperitoneum with carbon dioxide gas at 10 mmHg pressure. The Verese needle was then pulled out and a 5.5 mm trocar with canula was inserted in the same port.

The trocar was removed from the canula and a laproscope (5.5 mm diameter, Pro MIS, Frontline Limited, Carl-Zeiss-Strasse 3, Quickborn, Germany) connected to a light source was inserted through this canula. The female genitalia were visualized clearly near the urinary bladder and all reproductive disorders could be clearly seen by laparoscopy.

The following reproductive anomalies were observed on laparoscopic examination of the genitalia of the female rhesus macaques (Figure 1 A-G).

Ovarian cyst: a structure on the ovaries measuring over 2 cm and containing fluid is termed as ovarian cyst. The cyst is formed if a follicle

fails to release the egg, fluid remains inside it and this leads to the formation of a cyst.

**Ectopic pregnancy:** the condition in which the developing embryo implants outside the uterine cavity. An ectopic pregnancy develops because of a condition that slows the movement of egg or blocks its entry from the fallopian tube into the uterus. There are mainly two types of ectopic pregnancies: tubal and abdominal. In a tubal ectopic pregnancy, fertilized oocyte remains in the oviduct while in an abdominal ectopic pregnancy, gestation is completed in the peritoneal cavity.

**Ovarian tumor:** an abnormal or uncontrolled growth arising from within the ovaries. Epithelial tumors arise from the outer layer of the ovaries and germ cell tumors develop from the germ cells of the ovaries.

**Uterine rupture:** when there is rupture of the uterine wall or myometrial wall. Uterine rupture mainly occurs during pregnancy under the influence of hormones.

**Uterus unicornis:** when only one uterine horn develops while the second one is not fully developed or is absent. A unicornuate uterus has a normal single vagina and fallopian tube. Incomplete fusion of the mullerian ducts leads to development of the uterus unicornis.

**Uterine tumor:** an uncontrolled growth of the tissues of the uterus. These may be benign or malignant, e.g. fibroids, myomas, leiomyomas and polyps.

**Uterine edema:** abnormal swelling of the uterus. This is mainly because of infections inside the uterine body. The most common examples are endometritis or pyometra, etc.

## Results

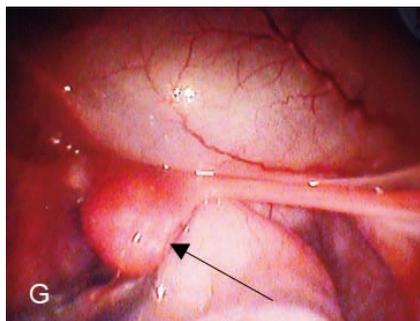
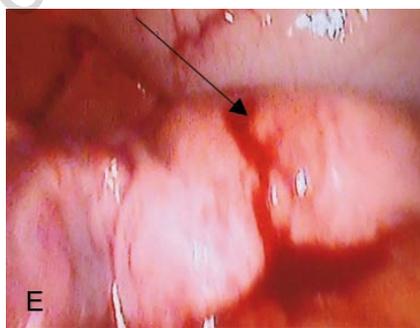
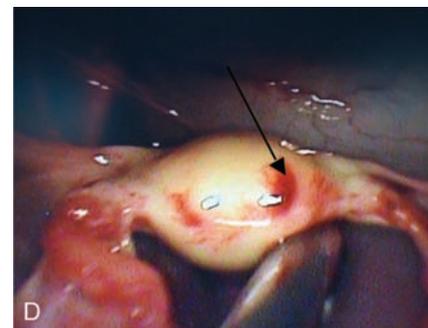
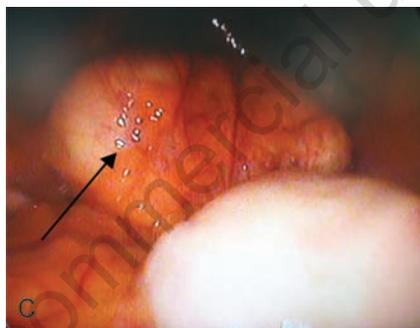
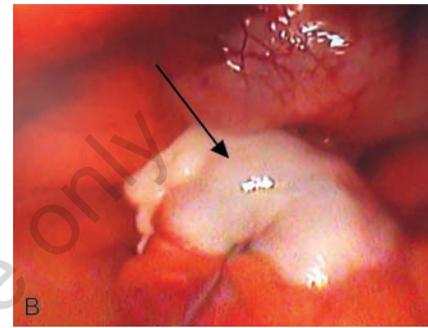
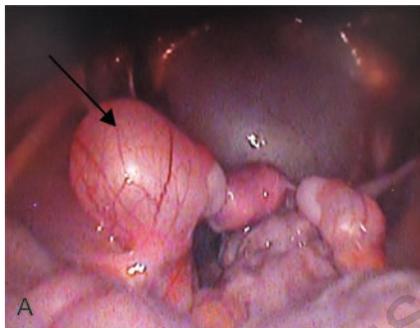
A total of 63 cases of gross reproductive abnormalities were observed during the laparoscopic examination of genitalia of 720 female rhesus macaques examined between July 2010 and June 2011. Total prevalence of the gross abnormalities was found to be 8.75% (63 of 720). The highest number of reproductive abnormalities was observed for ovarian cyst (3.05%) and the lowest was uterus unicornis (0.28%) (Table 2).

The highest number of cases of reproductive abnormalities was recorded in the 12-16 year old age group (33.33%) and the lowest in the 0-4 year old age group (3.17%) (Table 1). On laparoscopic examination of genitalia of 63 female rhesus macaques with reproductive abnormalities, only 52 animals were found pregnant during the breeding season from November to March in the 5-20 years of age group while 11 animals were found not to be pregnant; a pregnancy rate of 82.53% among female rhesus macaques with reproductive abnormalities.

## Discussion

Genital abnormalities have been found to play an important role in infertility by interfering in implantation and placentation.<sup>11</sup> Both in animals and in humans, reproduction outcome is directly correlated with genital abnormalities. In humans and non-human primates, these reproductive anomalies also reduce the viability of the offspring in various ways, such as abortions, stillbirths, or neonatal mortality.<sup>12-14</sup>

Sotirios *et al.* reported the prevalence of genital anomalies in human females to be approximately 6.0-7.4%; this is comparable to the present study on female rhesus macaques in which prevalence of genital anomalies was 8.75% (63 of 720). The difference may be because of the different diagnostic methods adopted; however, in the present study only laparoscopy was used. Laparoscopy is also considered to be one of the most efficient and accurate methods compared with other methods (hysteroscopy, 3D-ultrasound, sonohys-



**Figure 1. Reproductive abnormalities in free-ranging rhesus macaques. A) Ovarian cyst; B) ovarian tumors; C) ectopic pregnancy; D) uterine hemorrhages; E) uterine rupture; F) uterine cyst; G) uterus acornis.**

**Table 1. Uterine abnormalities according to age.**

Age (years)	N. reproductive abnormalities	Percentage (%)
0-4	2	3.17
4-8	10	15.87
8-12	16	25.39
12-16	21	33.33
16-20	14	22.22

**Table 2. Reproductive tract abnormalities observed by laparoscopy in free-ranging female rhesus macaques.**

Abnormalities	N.	Percentage (%)
Ovarian cyst	22	03.055
Ovarian tumors	12	01.666
Uterine tumors	9	01.250
Uterine edema	7	00.972
Uterine rupture	6	00.833
Ectopic pregnancy	5	00.694
Uterus unicornis	2	00.280
Total	63/720	08.750

terography) used to diagnose uterine anomalies.<sup>14</sup>

Reproductive outcome may also be affected by various intrinsic and extrinsic factors. Even the conditions in which the animals are housed may affect reproductive outcome in non-human primates.<sup>15-17</sup> A decrease in pregnancy rate (82.53%) in animals with reproductive anomalies has been reported in human females with uterine anomalies.<sup>18</sup> This shows that results of the reproductive anomalies in female rhesus macaques are close to those obtained in human females.

Ovarian cyst was found to be the most common genital abnormality (approx. 3.05%; 22 of 720). In a 2010 study by Marr-Belvin *et al.*, 4.8% of rhesus macaques were found to have ovarian cysts and ovarian neoplasms;<sup>19</sup> ovarian cysts alone accounted for 3.5% (16 of 458). Our results agree with these; in our current study ovarian cysts made up approximately 3.05% (22 of 720) of the genital abnormalities in the female rhesus macaques. There is a insignificant difference in incidence of ovarian cysts in the present study of free-ranging Indian rhesus macaques and in female rhesus macaques of Indian origin in the study by Marr-Belvin *et al.* 2010 (*data not shown*).

Ovarian tumors contributed approximately 1.66% (12 of 720) of the total uterine abnormalities. Dysgerminoma, teratoma, granulosa cell tumor, ovarian epithelial tumors, choriocarcinoma, have also been reported by various authors.<sup>20-23</sup> The highest reports of ovarian cancer have been reported in human females over the age of 50 years; incidence is 36.9 per 100,000 in the 30-50 year old age group, falls to

21 per 100,000 in the under 30 year olds, and in the women under 30 years it was 3 per 100,000.<sup>24</sup>

Uterine tumors made up 1.25% (9 of 720) reproductive abnormalities in all the animals examined. Studies in humans females reported 5.4-77% of women have uterine fibroid tumors of which 4% in the 20-30 year old age group,<sup>25</sup> 11-18% in the 30-40 year olds, and 33% in 40-60 year olds.<sup>26</sup> But there have been very little data reported for non-human primates.<sup>27</sup>

Uterine edema was seen in 0.97% (7 of 720) of the total uterine abnormalities. In female rhesus macaques, uterine edema may be associated with endometritis and pyometra.

Ectopic pregnancy was seen in 0.69% (5 of 720) of the total number of uterine abnormalities observed. Ectopic pregnancy has also been reported in non-human primates,<sup>28,29</sup> and a few cases of ectopic pregnancy in the uterine horn as well as in the abdominal cavity have been reported in rhesus macaques.<sup>30,31</sup> The prevalence of ectopic pregnancy ranges from 11.2 to 18.8 per 100 in human females.<sup>32</sup>

Uterine rupture was seen in 0.83% (6 of 720). Uterine tumors were previously reported in rhesus macaque and non-human primates.<sup>33,34</sup> In human females, the prevalence of uterine rupture ranges from 7.8 (symptomatic rupture) to 13 (asymptomatic rupture) per 1000. Uterus rupture is life threatening in all species and is associated with significantly higher maternal and perinatal mortality.<sup>35,36</sup> In human females, different authors have reported the average incidence of uterine rupture to range from 0.05% to 1.3%.<sup>37-40</sup>

In this study, the incidence of uterus unicornis was approximately 0.28% (2 of 720). Uterus unicornis has been observed in various animals,<sup>41-43</sup> but rarely reported in rhesus macaques. In human females, only one case in 76,000 of a pregnancy in the rudimentary horn has been reported and also in the present study,<sup>44</sup> uterus unocornis was the least common of all the reproductive abnormalities observed in free-ranging rhesus macaques.

It is very difficult to collect precise data regarding the prevalence of genital abnormalities in free-ranging wild animals as they are very difficult to capture from the wild and diagnosis of reproductive disorders is problematic. Many other abnormalities may be present in the reproductive tracts of the free-ranging rhesus macaques but this study only presents those reproductive abnormalities observed laparoscopically during the sterilization of 720 female rhesus macaques. The similarity in reproductive anomalies may be due to the fact that female rhesus macaques and female humans share a similar physiology. However, the difference in incidence may be due to the fact that these animals live in the wild and there may be many variations in environmental and genetic factors which may have an impact. One of the limitations of this study, is that none of the animals underwent in-depth laparoscopic examination, and histopathological testing of all the gross abnormalities in free-ranging female rhesus macaques would provide further useful data.

## Conclusions

The current study shows the similarities in reproductive abnormalities between female rhesus macaques and female humans. Laparoscopic diagnosis permitted the clear visualization and identification of the reproductive abnormalities in free-ranging rhesus macaques.

## References

- Gaitan H, Angel E, Sanchez J, et al. Laparoscopic diagnosis of acute lower abdominal pain in women of reproductive age. *Int J Gynaecol Obstet* 2002;76:149-58.
- Berker B, Mahdavi A, Shahmohamady B, Nezhat C. Role of laparoscopic surgery in infertility. *Middle East Fertile Soc J* 2005; 10:94-104.
- Medeiros LR, Fachel JM, Garry R, et al. Laparoscopy versus laparotomy for benign ovarian tumours. *Cochrane Database Syst Rev* 2005;20:4751.
- Sherry ER, Martin DC, Bowman RE, et al.

- Endometriosis in Rhesus Monkeys (*Macaca mulatta*) Following Chronic Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin. *Toxicol Sci* 1993;21:433-41.
5. Liao P, Bodkin N, Cho E, et al. Minimally invasive surgery via laparoscopy for intra-abdominal biopsy in obese rhesus macaques (*Macaca mulatta*). *Comp Med* 2004;54:159-64.
  6. Gaglio PJ, Baskin G, Bohm R Jr, et al. Partial hepatectomy and laparoscopic-guided liver biopsy in rhesus macaques (*Macaca mulatta*): novel approach for study of liver regeneration. *Comp Med* 2000;50:363-8.
  7. Elizabeth MS, Voe RSD, Valea F, et al. Medical and surgical management of reproductive neoplasia in two western lowland gorillas (*Gorilla gorilla gorilla*). *J Med Primatol* 2010;39:328-35.
  8. Kumar V, Raj A, Kumar P. Pregnancy diagnosis by laparoscopy in free range rhesus macaques (*Macaca mulatta*). *Open Vet J* 2011;1:3234.
  9. Rippey MK, Lee DR, Pearson SL, et al. Identification of rhesus macaques with spontaneous endometriosis. *J Med Primatol* 1996;25:346-55.
  10. Nichols SM, Bavister BD, Brenner CA, et al. Ovarian senescence in the rhesus monkey (*Macaca mulatta*). *Hum Reprod* 2005;20:79-83.
  11. Taylor E, Gomel V. The uterus and fertility. *Fertil Steril* 2008;89:1-16.
  12. Meredith F. Small reproductive failure in macaques. *Am J Primatol* 1982;2:137-47.
  13. Pedro A. Reproductive performance of women with uterine malformations. *Hum Reprod* 1993;891:122-6.
  14. Saravelos SH, Cocksedge KA, Li TC. Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. *Hum Reprod* 2008;14:415-29.
  15. Boot R, Leussink AB, Vlug RF. Influence of housing conditions on pregnancy outcome in cynomolgus monkeys (*Macaca fascicularis*). *Lab Ani* 1985;19:42-7.
  16. Bercovitch FB. Reproductive strategies of rhesus macaques. *Primates* 1997;38:247-63.
  17. Ha JC, Robinette RL, Sackett GP. Social housing and pregnancy outcome in captive pigtailed macaques. *Am J Primatol* 1999;47:153-63.
  18. Lin PC. Reproduction outcome in women with uterine anomalies. *J Women Health* 2004;13:33-9.
  19. Marr-Belvin AK, Bailey CC, Knight HL, et al. Ovarian pathology in rhesus macaques: a 12-year retrospective. *J Med Primatol* 2010;39:170-6.
  20. Eydelloth RS, Swindle MM. Intraductal mammary carcinoma and benign ovarian teratoma in a rhesus monkey. *J Med Primatol* 1983;12:101-5.
  21. Farman CA, Benirschke K, Horner M, Lappin P. Ovarian choriocarcinoma in a rhesus monkey associated with elevated serum chorionic gonadotropin levels. *Vet Pathol* 2005;42:226-9.
  22. Flinn RM. Serous cystadenoma of the ovary in a rhesus monkey. *J Pathol Bacteriol* 1967;94:451-2.
  23. Holmberg CA, Sesline D, Osburn B. Dysgerminoma in a rhesus monkey: morphologic and biologic features. *J Med Primatol* 1978;7:53-8.
  24. Yancik R. Ovarian cancer, age contrasts in incidence, histology, disease stage at diagnosis and mortality. *Cancer* 1993;71:517-23.
  25. Lurie S, Piper I, Woliovitch I, Glezerman M. Age-related prevalence of sonographically confirmed uterine myomas. *J Obstet Gynaecol* 2005;25:42-4.
  26. Lethaby A, Vollenhoven B. Fibroids (uterine myomatosis, leiomyomas). *Am Fam Physician* 2005;71:1753-6.
  27. Plesker R, Coulibaly C, Hetzel U. A spontaneous uterine sarcoma in a rhesus monkey (*Macaca mulatta*). *J Vet Med A Physiol Pathol Clin Med* 2002;49:428-9.
  28. McClure HM, Chang J. Ectopic pregnancy in a squirrel monkey. *J Am Vet Med Assoc* 1975;167:654-5.
  29. Schlabritz-Loutsevitch NE, Hubbard GB, Frost PA, et al. Abdominal pregnancy in a baboon: a first case report. *J Med Primatol* 2004;33:55-9.
  30. Jerome CP, Hendrickx AG. A tubal pregnancy in a rhesus monkey (*Macaca mulatta*). *Vet Pathol* 1982;19:239-45.
  31. Myers RE. The pathology of the rhesus monkey placenta. *Acta Endocrinologica Suppl* 1972;166:221-57.
  32. Storeide O, Veholmen M, Eide M, et al. The incidence of ectopic pregnancy in Hordaland County, Norway 1976-1993. *Acta Obstet Gynecol Scand* 1997;76:345-9.
  33. Cook AL, Rogers TD, Sowers M. Spontaneous uterine leiomyosarcoma in a rhesus macaque. *Contemp Top Lab Anim Sci* 2004;43:47-9.
  34. Kaspareit J, Friderichs-Gromoll S, Buse E, Habermann G. Adenocarcinoma of the Uterus in a common marmoset (*Callithrix jacchus*). *Primate Report* 2005;71:63-6.
  35. Ezechi OC, Mabayoje P, Obiesie LO. Ruptured uterus in South Western Nigeria: a reappraisal. *Singapore Med J* 2004;45:113-6.
  36. Gessesew A, Melese MM. Ruptured uterus-eight year retrospective analysis of causes and management outcome in Adigrat Hospital, Tigray Region, Ethiopia. *Ethiop J Health* 2002;16:241-5.
  37. Farmer RM, Kirschbaum T, Potter D, et al. Uterine rupture during trial of labor after previous cesarean section. *Am J Obstet Gynecol* 1991;165:996-1001.
  38. Lynch JC, Parady JP. Uterine rupture and scar dehiscence. A five-year survey. *Anaesth Intensive Care* 1996;24:699-704.
  39. Guise J, McDonagh M, Osterweil P, Helfand M. Systematic review of the incidence and consequences of uterine rupture in women with previous caesarean section. *BMJ* 2004;3:19-25.
  40. Zeteroglu S, Ustun Y, Engin-Ustun Y, et al. Eight years' experience of uterine rupture cases. *J Obstet Gynaecol* 2005;25:458-61.
  41. Brown JA, Hodder AD, Benak J, et al. Uterus unicornis in two mares. *Aust Vet J* 2007;85:371-4.
  42. Smith KC, Long SE, Parkinson TJ. Abattoir survey of congenital reproductive abnormalities in ewes. *Vet Rec* 1998;143:679-85.
  43. Iqbal M, Jafri SA, Khan SA. Incidence of gross reproductive disorders in female teddy goats in different age groups. *Pakistan J Agric Res* 1992;13:293-5.
  44. Utkin G, Jazayeri A. Diagnosis of rudimentary uterine horn in pregnancy. *Ultrasound Med* 2003;22:985-8.