Middle-East Journal of Scientific Research 8 (1): 46-51, 2011
ISSN 1990-9233

Ultrasonic Measurement of Follicle's Diameter and Bacteriological Examination During Foal Heat in Arabian Mares

Bouabdellah Benallou and Samia Meliani

Veterinary Institute of Tiaret, Ibn Khaldoune University, 14000 Algeria

Abstract: In this study, two factors influencing fertility in the foal heat were investigated with the aim to enhance effectiveness of reproduction management in Arabian mares. seventy three Arabian pure bred mares, aged from 3 to 20 years were used to examine the possibility to ultrasonically measure the follicle's diameter, in Arabian mares, during foal heat to predict the day of ovulation, according to follicle's size. 584 scans, were performed in animals belonged to the National Haras of Tiaret, Algeria. This study was carried out during the breeding period, corresponding to spring, of two years (2007 and 2008). The measurement of the follicles begun on the 7th day post partum, and the dominant follicle was followed. The day of ovulation was noted when follicle was not present on the ovary any more and it's conformed by the presence of a corpus luteum on this ovary three days later. No dominant follicle development was observed, in 11 mares (15,07%). The average diameter of follicles was 31,50 mm on the 8th day before the day of ovulation. On the day before ovulation, when the last measurement was performed, the average diameter of follicles was 43.16 mm, the evolution of the diameter average was about 1,67mm/day. Only forty nine mares were examined microbiologically, mares were randomly chousen , swabs were taken from clitorises. 71,42 % (n=35) of investigated mares were found to be bacteriologically positive and 28,58 % (n=14) of them were found negative. The most commonly isolated organisms were *Escherichia coli*, *Staphylococcus spp*. and *Pseudomonas aerogenosa* and the more frequent association of bacteria was *Escherichia coli* + *Staphylococcus spp.* and *E. coli* + *Pseudomonas aerogenosa*. The foal heat management is important to improve fertility in mares; ultrasonography and bacteriological analysis should be used in this regards.

Key words: Mare · Bacteriological · Gynecology · Reproduction · Foal Heat

INTRODUCTION

Mares exhibit seasonal reproductive activity, from spring till late autumn. During the reproductive periods, there is a 3 weeks cycle (21st to 23rd day), rarely shorter or longer [1]. A smaller number of mares show sexual activity during all year [2]. Transrectal manual palpation and ultrasonography are routinely performed determination of estrual phase in mares. According to the ultrasonic finding (nonechogenic, dark, oval structure), it is possible to determine the follicle on the ovary of mare in heat [3]. The tone of the uterus by rectal palpation during estrous is fair or poor (not tonic), but there are no specific changes at the time of ovulation [4].

Follicular size increases in diameter linearly over the seven days at an average rate of 2,7 mm per day [5, 6]; although England [3] reported that 24-48h before ovulation this increase stops. Preovulatory follicles exhibit a pronounced change in shape from a spherical to a conical or pear-shaped structure in 84% of the preovulatory periods, the remaining follicles retaining a spherical shape [5]. Furthermore, the degree of endometrial edema and the amount of luminal fluid generally decrease prior to ovulation, although this is not always the case [4]. It is possible to determine the stage of the mare's follicle, and according to finding to recommend the day of mating. By use of these methods it is possible to reduce the number of matting to 1 or 2, despite 4-5 (when not used).

Estrous period of the mare usually lasts 4-7 days, but is very variable, and usually ends approximately 24 hours (0-48 hours) after ovulation [4]. Ovulation is the culmination of a complex series of events, endocrine, biochemical and cytologic changes that result in the

Corresponding Author: Bouabdellah Benallou, Veterinary Institute of Tiaret, Ibn Khaldoune University, 14000 Algeria.
Tel: +213771956321, Fax: +21346425781.

collapse of the preovulatory follicle and the expulsion of the oocyte from the follicle [7]. Predicting the day of ovulation would have considerable use in co-coordinating the time of breeding, both by natural service or artificial insemination, with the expected time of ovulation [8]. Breeding after ovulation decreases the possibility of a successful pregnancy [9]. Equine practitioners use various symptomatic and exploratory parameters to determine the time of ovulation; nevertheless, the complexity of the ovulation mechanisms and the estrous duration, estrous symptomatology and time of ovulation variability are responsible that none of these parameters brings forward evidence with security that the ovulation is close. Because of the very long estrus in mares (5 to 7 days), and unpredictability of the day of ovulation it is necessary to develop method for predicting ovulation, with aim to perform as lower number of mating as possible [1].

Uterine infections have long been recognized as one of the major causes of reduced fertility in the mare. These infections are most often caused by opportunistic micro-organisms and a variety of species have been isolated [10]. The uterine infections often cause endometritis. Antibiotics are one component often used in the treatment of endometritis [11]. For clinicians there is a need of rapid microbiological diagnosis so that adequate treatment of the infection can be performed while the mare is still in oestrus [12, 13]. Therefore some mares are treated with antibiotics without a preceding microbiological investigation, whereas sometimes bacteriological cultivation is performed by the clinicians themselves. If the treatment is performed without a microbiological diagnosis, the choice of antibiotic is often based on data from earlier studies, e.g. Ginther [10]. However, the bacterial species isolated, as well as their susceptibility to antibiotics, may vary over time as well as from one population of horses to another [14].

Uterine infections of mares are known as an important cause for major economic loss in the equine industry [15, 16]. Detection of uterine disease and fertility controls include clinical, ultrasonographical, bacteriological, cytological and histopathological examinations [17]. Genital swab samples for microbiological examination are collected from the clitoris, cervix and uterus to screen microorganisms causing infertility. The most common bacteria isolated from mares with uterine disease are *Streptococcus zooepidemicus*, *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumoniae, Staphylococcus spp., Pasteurella spp., Taylorella equigenitali*s and fungal organisms (Candida and Mucor species) [18] . Bacteriological examinations should be supported by cytological and histopathological investigations, because the presence of inflammation is fundamental for the diagnosis and the prognosis [19]. Even in subclinical cases or in cases without any clinical signs, many microorganisms can be isolated from the uterus and smears contain leucocytes [20].

In Algeria, The National Haras mares had a reproduction management each year sine it's creation, ultrasonography were introduced for controlling genital tracts were introduced lately. In this work we examined the possibility to ultrasonically measure the follicle's diameter in mares during foal heat with aim to predict the day of ovulation, according to follicle's size, and we have investigate suspicious mares for bacteriological infections in the foal heat, with the aim to enhance reproductive management in Arabian mares.

MATERIELS AND METHODS

As experimental animals, 73 pure bred mares aged from 4 to 20 years, between during 2007 and 2008 were used. All mares belonged to the National Haras of Tiaret, Algeria. As well as to private owners whose mares were controlled for reproduction at the National Haras.

All foaling dates were known and follicular growth was controlled since the day 7 post partum, mares were examined by rectal palpation and ultrasound scanning, the status of the uterus and ovaries was determined, and the follicles diameters were noted daily sine ovulation, all mares were mated. Transrectal ultrasonography (FALCO 100, Pie medical) with 7, 5 MHz transducer for measurement of follicle' diameter in mares in estrus. This study was realized in reproduction period, corresponding to spring, of two years (2007 and 2008).

The measurement of follicles begun on the 7th day post partum, the dominant follicle was followed. The day of ovulation was noted when follicle was not present on the ovary any more and it's conformed by the presence of a lutea corpoa on this ovary three days after. For 73 mares 584 scans were performed.

For bacteriological examination, samples were collected from only 49 mare's clitoris using swabs, the specimens were submitted to the laboratory in the following two hours. The bacteriological examinations were cultured using: 5% blood agar, MacConkey medium, Agar agar medium, Schapman medium, King A medium. Samples were incubated for 24h at 37C°. Plates were incubated for 5days at 37C°. Routine methods were used to identify the germ.

RESULTS AND DISCUSSION

Follicular Measurement: In 73 examined mares, dominant follicle was observed and measured for 8 days before ovulation. The average of follicular diameters in mares and the evolution rates are reported in table 1. We observed no development of a dominant follicle in 11 mares (15,07%). The average diameter of follicles was 31,50 mm on the 8th day before the day of ovulation. On the day before ovulation, when the last measurement was performed, the average diameter of follicles was 43, 16mm. Follicular growth in all mares, according to the ultrasonic measurement of follicle's diameter showed that the rate of evolution was 1,67 mm/day. The evolution patterns are ported in Fig. 1.

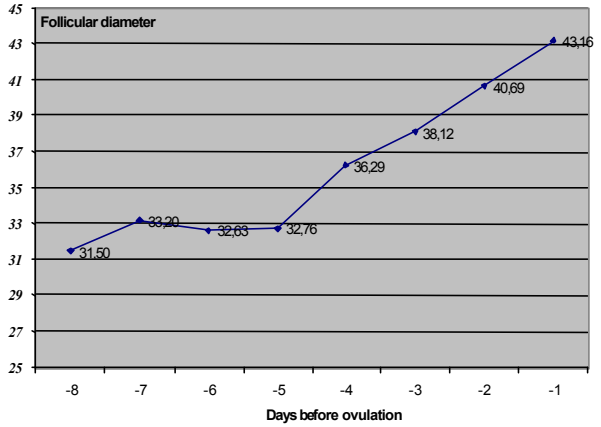

Fig. 1: Follicular evolution in Arabian mares.

The growth rate of follicle during estrous and mean diameter of the preovulatory follicle observed on day-1 was consistent with reported data [3, 8, 21, 22]. Follicles usually reach 4 cm in diameter before ovulating [4]. No preovulatory follicles are smaller than 35 mm or larger than 58 mm on day-1 [8]. The large preovulatory size variation between mares and estrous cycle would mean that this is a bad predictor of ovulation. However, the combination of a follicle that reachs 40 mm in diameter with a softening of this follicle seems to be the best predictor of ovulation.

As it was observed, several authors have described the softening of the preovulatory follicle in the mare, 90% of preovulatory follicles shows a change in consistency from turgid to soft during estrous [5, 23]. Few time before ovulation the follicle became more soft in 40% of mares [4, 5] and this finding is a good predictor of ovulation. The stepwise selection procedure demonstrates that ovarian texture is the most important parameter to determine ovulation.

Preovulatory follicles measured in mares had an average 41 mm diameter vertically, and 47,5 mm horizontally Mackintosh [24]. The average of 43 mm and 39 mm was also reported [25]. Slower follicular growth reported by Palmer and Driancourt [26] as 1,4 mm/day follicular growth rate in mares. According to interpolation of data mare should be mated when follicle of 38 to 42 mm is ultrasonically detected. Ovulation of such follicle will occur during 2 to 1 day with possibility of 95%.

The dominant follicle of major anovulatory waves generally does not reach a mean diameter comparable with the maximal diameter of preovulatory follicles [27]. The derived diameters representing follicle emergence in mare, 13 mm were at the reported peak of the wave-stimulating FSH surge in mares [28]. In major follicular waves, deviation occurs with development of a dominant follicle. The incidence of major anovulatory waves during an interovulatory interval has been reported as 24% in Quarter Horses [29]. Minor waves also develop in mares are characterized by a largest follicle that does not reach the diameter of a dominant follicle. The preovulatory diameter of the dominant follicle of the ovulatory wave is considerably larger in mares (e.g., 45 mm). In contrast, the preovulatory follicle is smaller in heifers (e.g., 16 mm) [27].

The ovulatory wave in mares emerges midway during an interovulatory interval of 22–24 days. After emergence, the follicles of a wave develop in a common-growth phase for several days [30]. At the end of the common-growth phase, a distinctive change in growth rates commences, wherein the developing dominant follicle maintains its growth rate, and the remaining follicles (subordinate follicles) grow at a reduced rate and regress. This process is called deviation and begins when the two largest follicles on average are 22,5 and 19,0 mm. The future dominant follicle emerges earlier on average than the future largest-subordinate follicle [31]. When the largest follicle was ablated at the expected beginning of

Table 1: Follicular diameters average and evolution rates in mares

Days before ovulation	-8	-7	-6	-5	-4	-3	-2	-1
Diameter average (mm)	31.50	33.20	32.63	32.76	36.29	38.12	40.69	43.16
Evolution rates (mm/day)	-	1.70	0.58	0.13	3.53	1.83	2.57	2.48

Table 2: The list of the organisms isolated from clitorises of 49 mares

Microorganism	n	*Rate (%)*
Negatives	14	*28,57*
E.Coli	9	*18,37*
Pseudomonas aerogenosa	7	*14,29*
Bacillus spp.	5	*10,20*
Staphylococcus spp.	4	*8,16*
Micrococcus spp.	5	*10,20*
Staphylococcus lentus	2	*4,08*
Staphylococcus Xylosus	2	*4,08*
Staphylococcus epidermidis	1	2,04
Total	49	100

deviation, the second-largest follicle continued to grow without interruption [28] and became dominant and ovulated in most mares.

Lieux [22] reported that preovulatory follicle is dominant 6 or more days before ovulation. Authors reported linear growth 99,5% of the follicle from the 7th day before ovulation. On that day, researchers measured average diameter of follicle of 29,4 mm. Preovulatory follicles reached in average 45,2 mm, with 2,7 mm/day growth rate.

A rate of 85% of the follicles was classified as non-spherical by day–1 to ovulation suggesting a relation between this observation and the softening of the preovulatory follicle [22]. It is possible that the softening of follicles is related with the elongation of the follicle to the ovulation cavity. The variation between days before ovulation observed in our study is in agreement with that report. However, the stepwise procedure did not include this parameter as a predictor of ovulation.

Bacteriological Sampling: Several germs were isolated simultaneously from 49 mares, the examinations showed different microorganisms isolated from clitorises in 71,42 % (n=35) examined mares, what is in agreement with observations of other authors [32, 34]. However, others reported 68% [10] of the mares yielded no significant growth. In those studies bacteria were found only in 36% of cases. Studies dealing with colonization of the uterus with bacteria in postpartum period and foal heat [32, 34, 36] demonstrated that type of bacteria and intensity of the infection after parturition changed in the uterine environment.

Table 2 shows the list of the organisms isolated from clitorises of 49 mares. The most commonly isolated organisms were *Escherichia coli, Staphylococcus spp*. and *Pseudomonas aerogenosa* and the more frequent association of bacteria was *Escherichia coli* + *Staphylococcus spp*. and *E. coli* + *Pseudomonas aerogenosa*.

The most commonly isolated organisms were *Escherichia coli, Staphylococcus spp.* and *Pseudomonas aerogenosa* and the more frequent association of bacteria was *Escherichia coli* + *Staphylococcus spp.* and *E. coli* + *Pseudomonas aerogenosa*. The list of isolated microorganisms is presented in table 2. Other studies have mostly been performed in normal populations of mares [10], which may be one explanation of this difference. But, also a study in barren mares [37] showed this dominance of ß-haemolytic *streptococci*. *Staphylococcus aureus* is reported to be a rather frequently isolated species from the equine uterus in a normal population of mares [10]. The bacterial species isolated may be influenced by the stud farm management and the breeding regime used. Also, the sampling technique influences the culture results. In the present study, most of the isolated *E. coli* and ß-haemolytic *streptococci* yielded moderate or abundant growth of the isolated bacteria, indicating that these isolates represented an infection in the uterus rather than a vulvovestibular contaminant [20, 38].

In post-partum mares, streptococci and coliforms are the most frequently found organisms; *E. coli* is the most typically isolated micro-organism found in early post partum, being replaced by streptococci during the advancing involution [35]. Anaerobic bacteria do not appear to be a problem in the post partum mare [33]. Although uterine swabs obtained from mares in foal heat are often bacteriologically positive, the incidence of positive samples is lower than shortly after foaling [36]. If only samples yielding heavy or moderate bacterial growth are considered to be meaningful, the number of positive mares in foal heat is small [33, 35]. However, it is obvious that the process of bacterial elimination is not completed

in all mares by the end of the foal heat. It has been suggested that healthy uterus during physiological anoestrus does not contain any bacteria [13]. During parturition and heat the uterine cervix is opened and tissues are swollen. These changes increase the possibility of uterine infection with both pathogen and non-pathogen bacteria [39]. The noted in this study of infected animals is very high percentage (71,42 %) in contrast to the results of other authors who found only 20-30% of infected mares [40, 41]. The reason for such numerous uterine infections in our experimental animals might have been poor environmental conditions, lower local and systemic resistance of the animals, as well as possibility of infections from bedding and hay. Low pregnancy rate in the examined animals could be caused by pathological uterine environment or post mating endometritis. It has been suggested that this phenomenon to be very important and can be one of the reasons for low fertility rate [42, 43]. Unfortunately, this aspect was not investigated in this study. Results obtained from other studies are discrepant. Hohenhaus and Bostedt [16] found that 39% of mares were pregnant, after the mating in foal heat, while , Sullivan *et al*. [44] achieved 70% of the fertility rate for the same period.

It was concluded that the foal heat management is important to improve fertility in mares; ultrasonography and bacteriological analysis should be used in this regards.

REFERENCES

1. Rossdale, P.D., 1992. Horse breeding, David & Charles, Newton Abot, Devon, Anatomy and physiology of reproduction in the mare, pp: 351-380.
2. Greenhoff, G.R. and R.M. Kenney, 1975. Evaluation of reproductive status of nonpregnant mares. J.A.V.M.A., (6): 449-458.
3. Palmer, E. and M.A. Driancourt, 1980. Use of ultrasound echography in equine gynecology. Theriogenol. (13): 203-216.
4. England, G., 1996. Allen's Fertility and Obstetrics in the Horse. 2nd ed. Blackwell Science, Oxford, London. pp: 241.
5. Ginther, O.J., 1979. Reproductive Biology of the Mare. Basic and applied aspects. Equiservices, Cross Plains, Wisconsin, USA. pp: 133-154.
6. Ginther, O.J., 1983. Sexual behaviour following introduction of stallion into a group of mares. Theriogenol., (19): 877-886.
7. Pierson, R.A., 1993. Folliculogenesis and Ovulation. In: Equine Reproduction. Angus, O., J. McKinnon, J.L. Voss, (Eds). Lea & Febiger. Philadelphia/London, pp: 161-171.
8. Ginther, O.J., 2000. Selection of the dominant follicle in cattle and horses. Anim. Reprod. Sci., (60): 61-79.
9. Koskinen, E., H. Linderberg, H. Kuntsi, L. Ruotsalainen and T. Katila, 1990. Fertility of mares after postovulatory insemination. J. Vet. Med. A., (37): 77-80.
10. Shin, S.J., D.H. Lein, A.L. Aronson and S.R. Nusbaum, 1979. The bacteriological culture of equine uterine contents, in-vitro sensitivity of organisms isolated and interpretation. J. Reprod. Fert. Suppl. (27): 307-315.
11. Perkins, N.R., 1999. Equine reproductive pharmacology Vet. Clin North Am. Equine Pract., (15): 687-704.
12. Ricketts, S.W. and M.E. Mackintosh, 1987. Role of anaerobic bacteria in equine endometritis. J. Reprod. Fertil., Suppl., (35): 343-351.
13. Ricketts, S.W., 1989. The barren mare. Diagnosis, prognosis, prophylaxis and treatment for genital abnormality. Part 1. In Practice, (11): 119-125.
14. Sternberg, S., 1999. Antimicrobial resistance in bacteria from pets and horses. Acta vet. scand. Suppl., (92): 37-50.
15. De Kruif, A., 1993. About the clinic and treatment of endometritis in the horse, I pathogenesis, symptomatology and diagnosis. Der Praktische Tierarzt., (74): 39-43.
16. Lieux, P., 1980. Comparative results of breeding on first and second post-foaling heat periods. 26th Am. Ass. Equine Pract., pp: 129-132.
17. Shideler, R.K., A.E. McChaseney, E.L. Squires and M.O. Sborne, 1987. Effect of uterine lavage on clinical and laboratory parameters in postpartum mares. Equine Practice, (9): 20-26.
18. Seyrek-intas, K., H. Bostedt, K. Herfen and D. Seyrekintas, 1997. Modified mare operative treatment and postoperative results pneumovagina'nin Caslick Method. Turk. J. Vet. Surg., (3): 10-15.
19. Belz, J.P. and Glatzel P.S. 1995. Fertility in mares after a disturbed as well as an undisturbed puerperium. Significance of histological and cytological examinations of the uterus. Tierärztl. Prax., (23): 267-272.

20. Waelchli, R.O., L. Corboz, and M. Doebeli, 1992. Streptomycin-resistent Escherichia coli as a marker of vulvovestibular contamination of endometrial culture swabs in the mare. Can. J. Vet. Res., (56): 308-12.
21. Ginther, O.J., 1986. Ultrasonic Imaging and Reproductive Events in the Mare. Equine services, Cross Plains, Wisconsin 53528, USA. pp: 378.
22. Pierson, R.A. and O.J. Ginther, 1985. Ultrasonic evaluation of the preovulatory follicle in the mare. Theriogenol., (3): 359-368.
23. Parker, W.G., 1971. Sequential changes of the ovulating follicle in the estrous mare as determined by rectal palpation. Proc. Ann. Conf. Vet. College Vet. Med. And Bio-Med. Sci., Colorado State University, Fort Collins, USA. pp: 149 -150.
24. Lindeberg, H., H. Kuntsi-Vaattovaara and T. Katila, 1992. Predicting ovulation in the mare 12th International Congress on Animal Reproduction The Hague, The Netherland, pp: 144-146.
25. Raun, K. and B. Synnestvedt, 1997. Folliculogenesis in mares - ultrasonographic aspects. Theriogenol., (47): 393.
26. Hohenhaus, M.U. and H. Bostedt, 1992. Criteria for evaluating, clinically and sonographically, the development of follicles up to single or double ovulation intra oestrum in the mare. 12th International Congress on Animal Reproduction The Hague, The Netherland, August 23th -27th 1992., 138-140.
27. Ginther, O.J., D.R. Bergfelt, M.A. Beg, C. Meira and K. Kot, 2004. In vivo effects of an intrafollicular injection of insulin-like growth factor 1 on the mechanism of follicle deviation in heifers and mares. Biology of Reproduction., (70): 99-105.
28. Gastal, E.L., M.O. Gastal, D.R. Bergfelt and O.J. Ginther, 1997. Role of diameter differences among follicles in selection of a future dominant follicle in mares. Biol. Reprod., (57): 1320-1327.
29. Ginther, O.J., 1993. Major and minor follicular waves during the equine estrus cycle. J. Equine Vet. Sci., (13): 18-25.
30. Ginther, O.J., M.A. Beg, F.X. Donadeu and D.R. Bergfelt, 2003. Mechanism of follicle deviation in monovular farm species. Anim. Rep. Sci., (78): 239-257.
31. Gastal, E.L., M.O. Gastal, M.A. Beg and O.J. Ginther, 2004. Society for Reproduction and Fertility DOI: 10.1530/rep.1.00259
32. Koskinen, E. and T. Katila, 1987. Uterine involution, ovarian activity and fertility in the postpartum mare. J. Reprod. Fert., Suppl. (35): 733-734.
33. Purswell, B.J., W.B. Ley, N. Sriranganathan and J.M. Bowen, 1989. Aerobic and anaerobic bacterial flora in the postpartum mare. Equine Vet. Sci., (9): 141-144.
34. Waelchli, R.O., M. Känzig, A. Gygax, L. Corboz and P. Rüsch, 1993. The relationship between cycle stage and results of uterine culture in the mare. Zentralb. Veterinarmed., (40): 569-575.
35. Katila, T., E. Koskinen, M. Oijala and P. Parviainen, 1988. Evaluation of the postpartum mare in relation to foal heat breeding. II. Uterine swabbing and biopsies. Zentralbl-Veterinarmed., (35): 331-339.
36. Saltiel; A., A. Gutierrez and C. de Buen-Llado Sosa, 1987. Cervico-endometrial cytology and physiological aspects of the post-partum mare. J. Reprod. Fert. Suppl., (35): 305-309.
37. Langoni, H., M.A. Alvarenga, F.O. Papa, C. Sakamoto, S. Baldini and F.J.P. Listoni, 1997. Aerobic, microaerobic and anaerobic bacteria in equine endometritis. Pferdeheilkunde, (13): 558-562.
38. Hinrichs, K., M.R. Cummings, P.L. Sertich and R.M. Kenney, 1998. Clinical significance of aerobic bacterial flora of the uterus, vagina, vestibule and clitorial fossa of clinically normal mares. JAVMA., (193): 72-75.
39. Morris, L.H.A. and W.R. Allen, 2001. Reproductive efficiency of intensively managed thoroughbred mares in Newmarket. Pferdeheilkunde, (17): 548-556.
40. Mackintosh, M.E., 1981. Bacteriological techniques in the diagnosis of equine genital infections. Vet. Rec., (108): 52-55.
41. Sullivan, J.J., P.C. Turner, L.C. Self, H.B. Gutteridge and D.E. Bartlett, 1975. Survey of reproductive efficiency in the Quarter-Horse and Thoroughbred. J. Reprod. Fertl. Suppl., (23): 315-318.
42. Troedson, M.H.T., 1997. Therapeutic considerations for mating-induced endometritis. Pferdeheilkunde, (13): 516-520.
43. Watson, E.D., 2000. Post-breeding endometritis in the mare. Animal Reprod. Sci., (60): 221-232.
44. Camillo F., P. Marmorini, S. Romagnoli, I. Vannozzi and M. Bagliacca, 1997. Fertility at the first post partum estrous compared with fertility at the following estrous cycles in foaling mares and with fertility in nonfoaling mares. J. Equine Vet. Sci., (17): 612-616.