Chapter 9

The Importance of Breeding Soundness Examination of the Mares

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Abstract

Reproductive efficiency is an essential factor for producing a healthy foal every year from broodmares. However, the reproductive physiology and seasonal pattern of mares restrict the reproductive efficiency and cause a limited breeding season. Moreover, there has been a universal birthday for all thoroughbreds on 1 January in the Northern Hemisphere. Thus, the owners aim to breed mares and birth foals as early as possible in the year. Breeding soundness examination is a systematic and effective procedure to assess the health and fertility status of the mares. An annual breeding soundness examination can save time, budget, and the welfare of the animal.

1. Introduction

The horses are one of the most valuable livestock animal species, used for agriculture, transportation, military operations, sports, and cultural activities for thousands of years. The accepted knowledge of the domestication of the horse occurred in Central Asia by the Turks and Mongols (Genç et al., 2022; Crossley, 2023; Peker et al., 2024). Selective breeding has significantly enhanced the evolution and development of current horse breeds worldwide, particularly in racing horses. The selection of broodmares for traits such as strength, racing performance, morphological structure, temperament, conformation, and other factors has improved the performance of different disciplines, including racing, showing, and dressage (Perdomo-Gonzales

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et al., 2024). The horse breeders' purpose is to produce one healthy foal each year from broodmares. However, the reproductive physiology and seasonal pattern of mares restrict the reproductive efficiency and cause a limited breeding season. Moreover, there has been a universal birthday for all thoroughbreds on 1 January in the Northern Hemisphere. Thus, the owners aim to breed mares and birth foals as early as possible in the year to give their foals a developmental advantage, enabling them to compete in their first race or be sold at higher prices (Crabtree, 2021). The *Breeding Soundness Examination* (BSE) procedure assesses the broodmare's fertility status, diagnoses, and solves the problems before breeding season that may interfere with breeding, conception, maintaining pregnancy, and foaling. Breeders can have healthy foals and save money owing to a thorough BSE procedure (Giffin and Darling, 2007; Usman et al., 2023).

2. Reproductive Physiology of the Mares

The equine species has a seasonal polyestrous long-day breeding pattern. Mares evolved to ensure that their foals are born into a favorable environment with optimal nutritional conditions, thereby maximizing their survival rate in nature. The breeding season in the Northern Hemisphere begins in the spring with an increase in daylight and temperature; the reproductive efficiency declines with a decrease in the amount of daylight. The official breeding season begins from 15th February to 15th June to support an equal official age for foals born in the same breeding season. However, almost 30 percent of the mares exhibit reproductive activity throughout the year (Nagy et al., 2000; Blanchard et al., 2003; Yüksel and Saat, 2017).

A circannual endogenous rhythm in mares characterizes the seasonality regulated by environmental factors, including photoperiod, nutrition, body condition, and temperature. Photoperiod is the primary factor that increased light signals, perceived by the retina, transmit to the pineal gland via the optic nerves, thereby decreasing the secretion of melatonin (Scott, 2020). Melatonin has an inhibitory function on the gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus, and this inhibitory effect disappears with the increased daylight. Pituitary gland, stimulated by increased pulsatile GnRH secretion, begins to release follicle-stimulating hormone (FSH), which induces follicular growth in the ovaries, and luteinizing hormone (LH), responsible for follicular maturation, estrogen secretion from follicles, ovulation, and luteinization. The estrogen produced in the follicles, particularly from the dominant follicle, promotes LH secretion through a positive feedback effect; meanwhile, estrogen and inhibin suppress FSH release from the pituitary gland via a negative feedback effect. The

ovulation of dominant follicle occurs with the LH surge, and progesterone secretion begins from the luteal cells of the corpus luteum (CL). The regular estrous cycles continue approximately every 21±2 days during the breeding season following the first ovulation of the year (Brinsko et al., 2011; Ekici, 2017).

The primary regulating factor of the seasonality is daylight length, which controls the duration of melatonin secretion. In the meantime, there are additional endogenous neuroendocrine mechanisms that affect the gonadotropic activity. The neurotransmitters function as suppressor agents on GnRH and/or gonadotropin secretion. Endogenous opioids inhibit gonadotropin secretion and control LH release during winter anestrus. Catecholaminergic neurons and dopamine also participate in the mare's reproductive activity by suppressing GnRH secretion (Nagy et al., 2000).

Nutrition has a significant impact on fertility, folliculogenesis and the timing of first ovulation in mares. The feed regime of the mare should be implemented depending on her age, breed, and reproductive status. Studies reveal that mares fed high-quality protein, vitamins, and minerals, and those with a positive energy balance, ovulate earlier after winter anestrus than mares fed a poor diet and those with a negative energy balance (Nagy et al., 2000). Nutritional supplementation of omega-3 fatty acids, L-arginine, and essential vitamins improves fertility (Khan et al., 2025). Pasture grazing also positively affects the ovarian activity and first ovulation in broodmares (Dugdale et al., 2010). Body condition score (BCS) is a valuable method for monitoring nutritional efficiency, energy intake, and reproductive performance by assessing overall and regional adiposity in animals (Henneke et al., 1983). The field trials documented that low BCS has a negative effect on fertility. The optimum BCS for entering the breeding season is accepted with a score of greater than 5 (on the scale 1 to 9) with a body fat content > 15% for early onset of ovulation. Inadequate energy intake and low BCS also adversely affect embryonic development and pregnancy. Studies reported that broodmares with a BCS between 6.5 and 8.0 may continue to ovarian activity through winter, and have a higher conception rate than mares with a ≤ 5 (Morley and Murray, 2014; Scott, 2020).

2.1. Seasonality in Mares

The reproductive rhythm of the mares is categorized into four seasonal periods: anestrus, vernal (spring) transition, breeding, and autumn transition phases. The reproductive year of the mare is gradually divided into those

four seasons in accordance with day length (Blanchard et al., 2003). In *the winter anestrus* phase, the mares are reproductively inactive after the shortest day of the year (winter solstice). During the deep anestrus period, ovaries are small, firm, and inactive; follicular development is minimal, with < 20 mm in diameter. The GnRH and LH secretions are at baseline levels; FSH concentration may be high due to the lack of negative feedback control of estrogen and inhibin. The plasma estradiol concentration is undetectable (< 5 pg/ml), and progesterone concentration is under 1.0 ng/ml. The mare is passive or unreceptive to the stallion and is disinterested in other mares. However, some mares may exhibit estrous behaviors and reproductive activity during the anestrus period, depending on the breed, nutrition, and other individual factors. The mare enters an anovulatory receptivity period with the vernal equinox, called the *spring (vernal) transition* period. With the increase in day length, the melatonin concentration decreases, and the suppressive effect of melatonin on the hypothalamus disappears, allowing GnRH secretion to increase. Ovarian activity begins with follicular development, and follicles greater than 20 mm in diameter may be detected on routine examinations. In the late transition phase, the follicular diameters reach ≥ 30 mm, but they don't ovulate because of inadequate LH synthesis and become regressing follicles. In the spring transition period, the GnRH release amplitude and frequency are gradually increased. Plasma FSH concentration is also increased early in this period, and it decreases one to two weeks before the first ovulation at the end of the period. LH concentration increases slowly and peaks just before the first ovulation of the year. Plasma estrogen levels are low during the early transitional phase, but begin to increase with growing dominant follicles 5-7 days before the first ovulation. The progesterone concentration is also low until after the first ovulation. The spring transitional period lasts one to two months, characterized by prolonged or irregular estrous signs (England, 2005; McCue et al., 2007).

The *breeding season* (i.e., the ovulatory phase or peak fertility period) of the year begins with the first ovulation following the spring transition period. The estrous activity occurs after the end of the luteal phase of the first ovulation. The estrous cycle of the mares lasts 21±2 days during the breeding season, thus described as seasonal polyestrous animals. The cycle can be divided into two phases: 1) estrus (follicular phase, sexual receptivity period with estradiol dominance), 2) luteal (early luteal phase forming corpora haemorrhagica, and late luteal phase having an active CL with progesterone dominance) phases. The length of estrus stage varies during the beginning and end of the breeding season. The shortest length generally occurs in late spring and summer (approximately 4-7 days) and may exceed 10 days in early spring

and autumn. The FSH stimulates follicular growth during diestrus stage, and estrogen concentration begins to rise following growth of recruited follicles. Pulsatile LH release stimulates the dominant follicle and oocyte maturation during the estrus phase, and ovulation occurs. LH concentration peaks two days after ovulation, and progesterone concentration increases along with the luteinization of granulosa cells. The diestrus stage lasts for 14-15 days, characterized by a high plasma progesterone concentration (>2 ng/ml). Endometrial release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) between days 13-16 post-ovulation causes luteolysis; subsequently, a rapid decline in plasma progesterone concentration occurs within 4 hours. The diestrus stage ends, and a new estrus cycle begins following progesterone decline. During the estrus phase, the mare is generally placid. Mares in heat straddle their hind legs and raise their tails when approached by the stallion, urinate, and evert their clitoris persistently. Some mares may show aggressive behaviors toward the stallion. In the luteal phase, the estrus signs terminate abruptly following ovulation. The aggressive response exhibit to the stallion (England, 2005; Ekici, 2017).

The peak breeding season ends with the decrease of day length, and the last ovulation of the year signals the beginning of the *autumn transition* period. The hypothalamic-pituitary-ovarian axis function declines due to the increase in melatonin secretion from the pineal gland. Inadequate gonadotropin secretion, suboptimal follicular and luteal development cause irregular estrous signs initially, and then acyclicity in mares (Irvine et al., 2000). Individual variations may be observed during the autumn transition period. Some mares display non-ovulatory follicular waves in decreasing magnitude, while others exhibit prolonged diestrus following luteinization (England, 2005).

3. Breeding Soundness Examination of the Mare

The purpose of the BSE is to check the mare's general and reproductive health, condition, and mating ability, potential of being pregnant, carrying, and delivering a healthy foal. If any morphological or infectious problem is diagnosed, it may be treated immediately before the breeding season. Examinations should be performed annually, preferably during the spring transition period, 50-60 days before the planned breeding season. Thus, the mare could be treated if she has any uterine infections, etc., and can breed as early as possible in the season (Giffin and Darling, 2007; Usman et al., 2023). The open mares (non-pregnant), mares have a reproductive pathology in the breeding season, barren mares after season, and mares with

embryonic loss, abortion, or different infertility history should also be added to the BSE practice (McCue, 2021).

Breeding soundness examination includes a systematic set of steps of breeding history evaluation, general physical examination, perineal conformation assessment, rectal palpation, transrectal ultrasonography, vaginal speculum examination, digital examination of cervix, uterus, and clitoral/vestibular cultures, and endometrial biopsy. Detailed diagnostic methods, such as transcervical endoscopic examination, hormone and chromosome analyses, and oviductal flushes, may be planned in addition to routine BSE tests for undiagnosed subfertile mares (Blanchard et al., 2003; Kılıçarslan and Uçar, 2017; Crabtree and Pycock, 2020).

3.1. Breeding History

The detailed breeding history assessment should begin with a proper identification of the mare. The breed, age, date of birth, registration name and number, color/markings, and microchip number should be recorded. The temperament, body condition, previous illness, surgeries, treatments, and vaccination administrations should be evaluated. The reproductive history should also be examined with questions about whether barren, maiden, pregnant, lactating, number of previous foals, abortions, dystocia, and number of previous breeding (stallion, artificial insemination, or embryo transfer) (Crabtree and Pycock, 2020; Usman et al., 2023). Young maidens may signify irregular cyclicity during their first estrous cycles or aggressiveness to the stallion. Barrens bred over two times but did not get pregnant, diagnosed as infertile, generally due to chronic uterine infections. The old mares (>15 years old) usually have low fertility because of multiple foaling and breeding, which causes uterine infections. The mares having a history of recurrent pregnancy losses should be screened in detail (Giffin and Darling, 2007).

3.2. General Physical Examination

The general physical examination is performed primarily to assess the ability of the mare to carry the foal to term. Any chronic disease history, including heart failures, and heaves, or heritable/genetic diseases such as patella luxation, degenerative joint disease, inguinal or umbilical hernia, laryngeal hemiplegia, and wobbler syndrome, is investigated during this procedure. Oral cavity, eyes, respiratory, musculoskeletal, and cardiac systems should be evaluated. Urine, hematology, serum biochemical, and fecal egg count analyses should be assessed (Brinsko et al., 2011; Usman et al., 2023). The serological tests of Coggins (equine infectious anemia) and equine

viral arteritis should be performed. The body condition score is a reliable indicator of a horse's health, indicating whether the horse is at an ideal weight, underweight, or overweight. It is usually hard to conceive of obese or overweak mares. Thus, the overweak mares should be supported with a high-energy diet that includes adequate protein, vitamins, and minerals. Obese mares should be put on a diet and an exercise program. Some barrens and maidens exhibit abnormal winter hair-coat (hirsutism) at the beginning of the season. The winter coat will be lost before the regular cycles start in healthy mares. However, mares with pituitary tumors may exhibit hirsutism (Giffin and Darling, 2007).

3.3. Perineal Conformation Assessment

The perineal conformation abnormalities directly affect fertility due to ascending genital canal infections. Therefore, the examination of the external genitalia primarily focuses on perineal conformation, as the anus and vulva should be completely vertical in this. The muscular tone of the vulva should be sufficient to close the vulvar entrance and to prevent air aspiration, which causes windsucking and pneumovagina in mares (Dascanio and McCue, 2021). The length and angle of declination of the vulva measure (Caslick's Index) to detect any necessity for surgical treatment (usually with Caslick's vulvoplasty technique) has been designed by Pascoe (1979).

Caslick's Index = Distance between dorsal commissure and pelvic brim (cm) x Vulva angle

The index <100 marks normal vulvar conformation, and no need to perform treatment; index between 100 and 150 marks to careful evaluation of the mare; the index >150 marks the mare obviously needs Caslick's vulvoplasty (Pascoe, 1979).

Any vulvar discharge, lateral or dorsal tears, Caslick's operation sutures to prevent pneumovagina, depigmented areas, and urine stain on the ventral commissure of the vulva should be noted. The clitoris examination shouldn't be forgotten in terms of clitoral enlargement, which indicates testosterone administration, pseudohermaphrodism, ovarian androgen-secreting tumor, or sex chromosome anomaly (Kılıçarslan and Uçar, 2017).

3.4. Rectal Palpation

Rectal palpation is an essential stage of BSE, in which a veterinarian may palpate the ovaries, uterus, and cervix in mares. The mare should be adequately restrained before examination to protect both animal and veterinarian. The ovaries are bean-shaped in mares. The size, presence or absence of follicles, and the stage of the estrous cycle can be confirmed between the fingers and

thumb. In the anestrous period, the ovaries are small in size and inactive; hypoplastic ovaries are also very small. The different-sized (>20 mm in size) follicles began to palpate during the transition period, and ≥35 mm in size dominant follicles can be examined during the breeding season. Increased ovarian size may indicate an ovarian tumor, hematoma, or parovarian cysts (Blanchard et al., 2003). The non-pregnant uterus is T- or Y-shaped, and its size, position, and tone can be evaluated by palpation. During the deep anestrous phase, the uterus is thin-walled and difficult to palpate; tonus begins to increase during the transition period. The uterus is edematous, thick, and heavy in the estrus stage of the cycle. Uterine pathologies, such as endometrial fold atrophy, lymphatic cysts, uterine neoplasia, and large purulent fluid in the lumen, can be detected by rectal palpation. The cervix examination per rectum reveals the stage of estrus. In the estrus stage, the cervix is dilated, soft, and flaccid; in the diestrus stage, it is tightly closed and thick-walled (Kılıçarslan and Uçar, 2017).

3.5. Transrectal Ultrasonography

Ultrasonography examination is beneficial for diagnosing reproductive tract pathologies, follicular dynamics, ovulation and development of CL, pregnancy, and twins. Doppler ultrasound is an advanced technology of grey-scale ultrasound. The uterine and ovarian blood flow can be evaluate during estrous cycle and pregnancy (Ortega-Ferrusola et al., 2022). Fluid-filled, anechogenic, various-sized follicles may be observed in the ultrasound. A conic or pear-shaped preovulatory follicle with a diameter of 40-50 mm can be observed 24 hours before ovulation. Ovulation can be detected as the absence of a preovulatory follicle in the ovulation fossa. Corpus hemorrhagicum is seen as a homogeneous, grey image, while CL has a more echogenic appearance in ultrasonography. The estrus stage has a dramatic ultrasonography image with a small amount of free fluid in the uterine lumen and oedema due to elevated estrogen. The cross-section of the uterine horn is described as a "sand dollar" or "spoke wheel" image (McCue, 2021).

3.6. Vaginal Speculum Examination

Visual vaginal examination is a useful method to evaluate the entire vagina and the external opening of the cervix. The determination of the stage of the estrous cycle, cervix or vaginal wall lacerations, accumulation of purulent discharge or urine in the vagina, vaginal or cervical inflammations, persistent hymen, adhesions can be performed by visual examination per vaginam. However, the vaginal examination has an important risk of genital

tract contamination by air and pathogens into the uterus. Thorough cleaning of perineum with proper disinfectant solutions, and wrapping tail before examination should not be forgotten (Brinsko et al., 2011).

Vaginal examination using a sterile speculum helps to evaluate various physiological and pathological conditions in mares. In the estrus stage, the vaginal mucosa is pink, hyperemic, bright, and moist. The cervix is lying on the vaginal floor, relaxed, soft, and pink colored during estrus. In the diestrus stage, the vaginal mucosa is dry, grey to pale pink, and the cervix is located well off the vaginal floor, closed, and firm. In the anestrus, the vaginal mucosa and cervix are dry and pale; the cervical os is closed and located high on the vaginal vault (Kılıçarslan and Uçar, 2017; Dascanio, 2021a).

A complete or partial persistent hymen may be detected between vestibule and in the maiden mares during vaginal examination. The persistent hymenal tissue blocks the drainage of endometrial secret and may lead a uterine infection, it should be removed manually or surgically two or three weeks before breeding (Dascanio, 2021b). Various colored and consistent (white, grey, watery, or purulent) fluid on the floor of the vagina coming from the cervix reveals a uterine infection. Yellow, urea-like odour fluid pooled near the cervical opening is urine, which is diagnosed as urovagina, another cause of infertility (Giffin and Darling, 2007).

3.7. Digital Examination of the Vagina and Cervix

Manual examination of the vagina and vulva is performed following a complete vaginal speculum exam during BSE. The digital examination should be done using maximum sterile technique and gently to avoid ascending contamination and damage. Postpartum diagnosis of vaginal and cervical trauma, laceration due to dystocia or uneventful delivery, retained fetal membranes, and adhesions can be performed. The cervical lumen may be controlled by gently dilating and palpating with a finger to explore lacerations and adhesions (Sitters, 2021).

3.8. Endometrial Biopsy

An endometrial biopsy is used to diagnose the cause of pregnancy loss and uterine diseases, usually for barren mares. The uterine tissue sample is mostly collected prior to the onset of breeding season, in the diestrus or early estrus stage. The tissue samples may also be used for uterine culture, cytological evaluation or molecular analyses such as reverse transcription polymerase chain reaction (RT-PCR). Histopathological analysis of the tissue sample

reveals pathological changes in the endometrial glands, including luminal and glandular cells, as well as inflammatory or degenerative changes, and the accumulation of inflammatory cells, such as polymorphonuclear leukocytes (Crabtree and Pycock, 2020; McCue, 2021; Usman et al., 2023).

3.9. Endometrial Cytology

Cytological sampling from the uterus is a simple, practical, and helpful method for the detection of endometritis. Uterine culture should be performed in conjunction with endometrial cytology. Uterine cytology samples may be obtained by swab, brush, or a low-volume uterine lavage method from the uterine cavity and endometrial surface. The sampling should be under sterile conditions to avoid contamination of the genital tract. The stained cytology preparations are investigated under a microscope for neutrophils, microorganisms, and epithelial cells. Samples from mares having acute and subacute endometritis contain increased neutrophils and degenerated epithelial cells. An excessive number of macrophages, lymphocytes, and plasma cells is observed in the mares having chronic endometritis. The moderate to severe content of debris is also associated with bacterial endometritis (Dascanio and Ferris, 2021). The infections caused by yeast and fungi may be diagnosed with uterine cytology, due to the proliferation of these organisms in the luminal epithelium and uterine lumen (Brinsko et al., 2011).

3.10. Clitoral and Uterine Culture

Clitoral culture is mostly used to diagnose venereal diseases caused by *Klebsiella pneumonia*, *Pseudomonas aeroginosa*, and *Taylorella equigenitalis*. The stallion could be contaminated with those pathogens from an asymptomatic mare during breeding (Giffin and Darling, 2007). Uterine cultures may be obtained via uterine cytology swabs, brushes, lavages, or biopsy samples. The most common pathogens of the uterus are *Streptococcus zooepidemicus, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Candida* spp., and *Aspergillus* spp (Kılıçarslan and Uçar, 2017; Dascanio, 2021).

3.11. Other Diagnostic Tests

The advanced diagnostic procedures can be performed in addition to the routine breeding soundness examination in undiagnosed infertile mares. Karyotype analyses in maiden mares, gonadal hormones (progesterone, estrogen, and testosterone) for the detection of ovarian structure functions or granulosa cell tumor, hysteroscopy, and oviductal flush or PGE2 application

to evaluate any blockage of the oviduct could be performed in broodmares (Blanchard et al., 2003; Crabtree and Pycock, 2020).

4. Conclusions

The broodmares should be healthy and reproductively productive to meet the demands of breeders. The general health and the reproductive system organs should be controlled annually before the expected breeding season. Breeding soundness examination is a systematic and effective procedure to assess the health and fertility status of the mares. Annual breeding soundness examination can save time and budget as well as the welfare of the animal.

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