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# Effect of the inseminate and the site of insemination on the uterus and pregnancy rates of mares<sup>☆</sup>

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#### Abstract

In this review, effects of the composition of the inseminate on uterine response and pregnancy rates in mares are discussed. The inseminate can differ for volume, sperm concentration, total sperm numbers, presence, absence, or proportion of seminal plasma, and extender composition. Semen can be used as fresh, cooled, or frozen. The site of semen deposition also plays a role; semen is deposited either into the uterine body (standard artificial insemination (AI)) or into the tip of the uterine horn ipsilateral to the preovulatory follicle (deep AI) using the hysterocopical or transrectally guided techniques. In addition to pregnancy rates, some uterine responses to the inseminate are considered including myometrial contractions, transport and elimination of sperm, and uterine inflammation, which is reflected as numbers of polymorphonuclear leukocytes, enzyme levels, and presence of intrauterine fluid. Reproductively normal and abnormal mares are compared.

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#### 1. Introduction

Artificial insemination (AI) is a widely practiced breeding method in most sport horse breeds and in many countries. In the natural breeding, the major part of the ejaculate gains access into the uterus without human intervention, but in AI the composition of

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the inseminate can be modified in many ways. We can fractionate the ejaculate and use only the first sperm-rich jets; we routinely discard the gel fraction, which is released towards the end of the ejaculate. We can remove seminal plasma totally or reduce its proportion by dilution of semen. Compositions of seminal extenders are various, but this review does not discuss pregnancy rates in this connection. Dilution with semen extenders also decreases sperm concentration. The inseminate volume has a wide range, commonly from 0.2 to 100 mL. The sperm dose has a wide range as well, nowadays, usually from 5 to  $500 \times 10^6$ progressively motile sperm. The standard site of semen deposition is the uterine body, but in conjunction with the use of sex-sorted semen and low semen doses the deep AI has been adopted. Semen is deposited into the tip of the uterine horn ipsilateral to the preovulatory follicle using transrectal guidance or on the papilla of the uterotubal junction when using the hysteroscopic method. Finally, semen can be used fresh immediately after collection, or it can be extended and cooled allowing shipments up to 48 h, or it can be cryopreserved in liquid nitrogen. This review does not compare pregnancy rates related to different semen storage methods, but limits to the uterine effects of different types of semen.

With the above listed modifications of the inseminate we aim to higher pregnancy rates or reduced costs. Cooling and cryopreserving semen reduces costs and potential health hazards incurred by transporting animals and provides easier access to genetically valuable material. It is extremely difficult and expensive to carry out AI trials with adequate numbers of mares and stallions to compare different methods. Fertility trials have been performed, but the results have to be viewed consciously because of the small numbers of horses in the experimental groups. It will be difficult to know the commercially optimal number of sperm in an AI dose due to differences among stallions.

Pregnancy rate is the final outcome of AI, but effects of the inseminate on uterine response have also been studied, e.g. on uterine contractions, transport and elimination of sperm, and on uterine inflammation reflected as numbers of polymorphonuclear leukocytes (PMN), enzyme levels, or amounts of ultrasonically visible intrauterine fluid collections. When looking at the uterine response, the type of the mare has to be taken into account. Mares have been categorized as reproductively normal or abnormal based on age, previous breeding history, endometrial biopsy category, or susceptibility to uterine infections.

## 2. Insemination volume, sperm concentration and total sperm numbers

Insemination volume is usually 5–50 mL of fresh semen, but if sperm concentration and motility are very low, higher volumes have to be used. Rowley et al. (1990) reported AI volumes of 100 and 200 mL to be associated with lower embryo recovery rates than the volume of 10 mL. However, sperm concentrations in large volumes were much lower (2.5, 1, or  $0.5 \times 10^6 \,\mathrm{mL^{-1}}$ ) than in 10 mL (10 or  $25 \times 10^6 \,\mathrm{mL^{-1}}$ ). In fact, Jasko et al. (1992) showed that adequate sperm concentration is more important than the volume. Mares inseminated with 10 or 50 mL had high embryo recovery rates (70–80%), when concentration was  $25 \times 10^6 \,\mathrm{sperm/mL}$ , but only 35–60% with the concentration of  $5 \times 10^6 \,\mathrm{sperm/mL}$ . Excessive dilution may be detrimental to sperm transport and fertilization; although adequate sperm numbers are available, a minimum sperm concentration may be necessary (Squires

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