# LOW-DOSE INSEMINATION

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A normal fertile stallion will deposit anywhere from 2 to 10 billion spermatozoa or more into the reproductive tract of a mare after ejaculation. Only a few thousand sperm actually make it into the oviduct and even fewer are present in the central (ampulla) portion of the oviduct where fertilization of the ovulated egg occurs. A majority of the ejaculate is expelled from the uterus within 10 to 20 minutes after mating through the open cervix by uterine contractions. Most of the sperm remaining in the uterus are engulfed and destroyed by white blood cells as part of the normal post-mating inflammatory response.

In an artificial insemination program, it is generally accepted that the standard minimum insemination dose for mares is 500 million progressively motile spermatozoa. The optimal concentration of spermatozoa for insemination is between 25 and 50 million sperm per ml. As a consequence, a typical insemination volume of freshly collected semen mixed in a traditional semen extender may be approximately 20 ml. Insemination with less than 100 million spermatozoa using traditional techniques will result in decreased pregnancy rates per cycle.

In some instances it may be desired or necessary to inseminate a mare with a lower than usual number of sperm. This may be the case when a limited number of frozen semen straws are available, if a stallion has a low sperm count in his ejaculate, or if the book of mares for a given day are higher than the amount that may be bred with a standard insemination dose.

Recent studies have indicated that acceptable pregnancy rates can be achieved using what has been termed ‘low-dose insemination techniques’. The number of spermatozoa deposited using low-dose protocols range from less than 5 million to 50 million or more spermatozoa in volumes ranging from less than a tenth of a milliliter up to one or two milliliters. Obviously, the intent would be to inseminate a mare with as many motile sperm as possible, but in some situations the numbers just are not there. It may be necessary to centrifuge the semen to concentrate the spermatozoa if the original ejaculate is dilute.

The most common low-dose technique used on breeding farms and veterinary clinics is a manual deep-horn procedure that does not utilize sophisticated or expensive equipment. In fact, the only equipment required for the procedure is a flexible insemination pipette. First, the preovulatory follicle is identified as being on either the left or right ovary. Next, a small volume of semen is drawn up into the tip of the pipette and the pipette is passed through the cervix into the uterine

body using a traditional initial approach. Then, the arm of the person performing the procedure is removed from the vagina and introduced into the rectum and the location of the pipette tip is determined. The uterus is subsequently gently manipulated and the pipette is passed up the uterine horn adjacent to the ovary containing the preovulatory follicle. The pipette is advanced until the tip is located at the end of the uterine horn near the uterotubular junction or entrance to the oviduct. The semen is then deposited and the pipette withdrawn from the mare.

In a few instances it may be beneficial to sedate the mare or to administer a medication such as Buscopan® to facilitate manipulation of the pipette via rectal palpation. In most instances the mare will be administered an ovulation inducing agent such as human chorionic gonadotropin (hCG), deslorelin, or equine luteinizing hormone prior to or at the time of deep-horn insemination.

The second technique for low-dose insemination is hysteroscopy. With this procedure, the mare is sedated, an endoscope is passed through the cervix, and the uterus is inflated with air. The scope is passed up the horn adjacent to the ovary containing the preovulatory follicle. A catheter containing a small volume of semen is passed through a tube within the scope and out the end of the scope. The uterotubular (oviductal) junction is visualized and the catheter tip is moved so that the small volume of fluid containing spermatozoa is deposited directly onto the oviductal opening. The primary advantage of the hysteroscopic technique is direct visualization and precise deposition of semen onto the oviductal junction. Disadvantages include the initial cost of the videoendoscope, time required to set-up, perform and clean-up after the procedure, and the requirement for multiple trained personnel.

Controlled research studies have reported that there are no significant differences in pregnancy rates between the manual deep-horn technique and the hysteroscopic procedures. Both methods can yield acceptable pregnancy rates using low numbers of spermatozoa if the stallion has good inherent fertility. However, low-dose insemination techniques may have limited effectiveness in subfertile or infertile stallions. Please consult with your equine veterinarian for additional information on the various low-dose insemination options.