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Effect of sperm number and site/technique of insemination on pregnancy in mares

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Summary

This survey reviews data currently published in literature with respect to the effects of sperm number and different artificial insemination (AI) techniques (e.g.: routine insemination into the uterine body, rectally or ultrasonographically guided deep intracornual insemination ipsilateral to the preovulatory follicle, hysteroscopic insemination onto the uterotubal junction ipsilateral to the preovulatory follicle) on the pregnancy rate in mares. It is concluded that a single insemination close to ovulation with reduced numbers of cooled [~25 - 100 x 10^6 progressive motile spermatozoa (pms) instead of ~300-1.000 x 10^6 pms] or frozen/thawed [~25 - 100 x 10^6 pms postthaw instead of either a total of 800 x 10^6 frozen spermatozoa with a post thaw motility of Δ 35% or 300 x 10^6 pms] semen from fertile stallions may achieve pregnancy rates similar to commercial doses (Minimum Standard requirements of WBFSH) under optimum conditions. However it remains questionable, whether or not this similarity can be attributed to the use of low dose insemination techniques. Sperm doses Δ 25 x 10^6 pms post-thaw, when inseminated deep intrauterinely with the help of a videoendoscope hold no advantage over conventional uterine body AI. When inseminating with <10 x 10^6 pms post-thaw the mares should be inseminated hysteroscopically. This amount of spermatozoa is therefore considered to be close to the critical sperm number. When only very low numbers of spermatozoa (e.g. sex-sorted spermatozoa) are available the critical sperm dose seems to be close to 5 x 10^6 spermatozoa and should be transfered hysteroscopically rather than by rectally or ultrasonographically controlled deep intrauterine insemination to give the best results. Washing of spermatozoa preceeding insemination to remove damaged spermatozoa and debris seems to have no beneficial effect on pregnancy rates when low doses of spermatozoa are transferred hysteroscopically. Neither does preinsemination intrauterine treatment with prostaglandin E2.This hormone influences oviductal smooth muscle function and subsequent oviductal sperm colonization. Giving prostaglandin E2 prior to low dose transrectally-guided or hysteroscopic-guided deep intrauterine insemination was not seen to increase pregnancy rates in mares. Twin pregnancy rates may be lower after contralateral double ovulations when mares are inseminated deep into the uterine horn on the side of the major preovulatory follicle. Rectally guided deep intrauterine insemination technique may be more feasible for practitioners in the field when inseminating low doses of spermatozoa, but there may be a higher risk of uterine trauma compared to hysteroscopic AI. On the other hand, hysteroscopic AI is an expensive technique and requires skilled operators. In mares with an overlarge uterus or cases of excessive endometrial edema (foal heat) it could become impossible to locate the uterotubal junction (UTJ). Nevertheless, health risks associated with deep intracornual insemination warrant its further research before extensive use. Application of low dose insemination techniques for less fertile stallions is highlighted in the literature but its usefulness is debatable and further research is required into stallion sperm parameters that may or may not be compensable by the insemination technique.

Keywords: low dose insemination; stallion semen, hysteroscopic insemination, sperm number, fertility

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Einfluss der Spermiendosis und der Lokalisation der Spermadeponierung bzw. Technik der Samenübertragung auf das Trächtigkeitsergebnis bei Stuten

Dieser Übersichtsartikel fasst die bislang veröffentlichten Daten zusammen, soweit sie sich auf Effekte der Spermiendosis und der Besamungstechnik (konventionelle Samenübertragung in den Gebärmutterkörper, rektal oder sonographisch geleitete tief intracornuale Besamung ipsilateral zum präovulatorischen Follikel, hysteroskopisch geleitete Samenübertragung auf die uterotubale Verbindung ipsilateral zum präovulatorischen Follikel) auf das Trächtigkeitsergebnis bei der Stute beziehen. Zusammenfassend lässt sich feststellen, dass Einzelbesamungen in zeitlicher Nähe zur Ovulation mit reduzierten Spermienzahlen flüssigkonserviertem [~25 - 100 x 10^6 progressiv motile Spermien (pms) im Vergleich zu kommerziell empfohlenen Spermiendosen ~300-1000 x 10^6 pms] oder tiefgefrorenem [~25 - 100 x 10^6 pms im Vergleich zu kommerziellen Richtwerten: 800 x 10^6 mit einer Auftaurate von Δ 35% pms oder 300 x 10^6 pms] Hengstsperma von fertilen Hengsten unter optimierten Besamungskonditionen Fruchtbarkeitsresultate erzielbar sind, welche sich von den Ergebnissen nach Besamung mit ausreichend dosiertem kommerziellem Sperma (Richtwerte WBFSH) nicht deutlich unterscheiden. Ob diese Ergebnisse auf Effekte der Besamungstechnik zurückzuführen sind ist nicht ausreichend geklärt. Wird eine Tiefgefrierspermadosis von Δ 25 x 10^6 pms eingehalten scheinen keine Vorteile der hysteroskopischen im Vergleich zur konventionellen Samenübertragung zu bestehen. Wird Tiefgefriersperma mit <10 x 10^6 pms versamt sollte ausschließlich die hysteroskopische Methode eingesetzt werden; so dass diese Dosis als kritische Spermiendosis angesehen werden könnte. Sind nur sehr geringe Spermienzahlen verfügbar (z.B. gesextes Sperma) so scheint die kritische Spermiendosis bei 5 x 10^6 pms zu liegen, wobei tiefintracornual und bevorzugt hysteroskopisch besamt werden sollte. Das Waschen von Spermien mit speziellen Separationstechniken zur Entfernung von Zelldetritus und geschädigter Spermien präinseminationem hat keinen positiven Effekt auf das Trächtigkeitsergebnis bei hysteroskopischer Samenübertragung. Ebensowenig ließ sich präinseminationem durch prätubale Applikation von Prostaglandin E2 – zum Zwecke der Beeinflussung der glatten Muskulatur des Eileiters – das Trächtigkeitsergebnis nach rektal geleiteter tiefintracornualer oder hysteroskopischer Besamung verbessern. Zwillingsträchtigkeitsraten bei contralateraler Doppelovulation erschienen reduziert nach tiefintracornualer Besamung ipsilateral zur Seite des größten präovulatorischen Follikels. Bei Einsatz deutlich reduzierter Spermienzahlen erscheint die rektal geleitete tief intracornuale Besamung hinsichtlich der Praktikabiltät für den Feldeinsatz besser geeignet, birgt aber im Vergleich zur hysteroskopischen Besamung ein höheres Risiko uteriner Traumatisierung. Andererseits bedarf die hysteroskopische Samenübertragung teurer Instrumentarien und erfahrener Operatoren. In Fällen vergrößerter Uteri und ausgeprägtem endometrialem Ödem (Fohlenrosse) gelingt die visuelle Darstellung der uterotubalen Verbindung gelegentlich nicht. Risiken

hinsichtlich hygienischer Aspekte bei Einsatz tiefintrauteriner Besamungstechniken bedürfen weiterer Untersuchungen. Der Einsatz tiefintrauteriner Besamungstechniken bei Hengsten mit vorberichtlich herabgesetzten Fruchtbarkeitsraten wird in der Literatur kontrovers diskutiert und bedarf weiterer Untersuchungen hinsichtlich durch die Besamungstechnik potentiell kompensierbarer spermatologischer Parameter.

Schlüsselwörter: Besamung mit geringer Spermienzahl, Hengstsperma, hysteroskopische Samenübertragung, Spermiendosis, Fruchbarkeit

Sperm number

Commonly 500 x 10^6 progressively motile spermatozoa (pms) provide satisfactory pregnancy rates when mares are inseminated every other day during estrus (*Pickett* and *Voss* 1975). A dose of 50 x 10^6 pms can be regarded as a critical sperm number for insemination with fresh semen as reported by *Householder* et al. (1981). The most commonly used dose is 500 x 10^6 pms with fresh semen, recommended by *Pickett* et al. (2000) for maximum reproductive efficiency. These authors suggested 1.000 x 10^6 pms for cooled semen at the time when the semen is packaged prior to cooling, and 800 x 10^6 total frozen spermatozoa with a post thaw pms percentage of at least 30 %. Others recommend insemination with 300 x 10^6 pms frozen/thawed spermatozoa (*Leipold* et al. 1998) close to ovulation or insemination daily until ovulation with a total of 400 x 10^6 frozen/thawed spermatozoa (*Vidament* et al. 1999). Although the minimum number of spermatozoa per insemination dose probably varies between stallions, the optimal amount required with frozen/ thawed spermatozoa has not yet been determined for the stallion.

Few studies exist that provide fertility data from a large number of mares inseminated with frozen/thawed semen. *Müller* (1987) reported in a five year survey (1981-1985) a 43% (414/959) first cycle pregnancy rate, when mares were inseminated with 300 x 10^6 pms in an average of 2,2 times per cycle. *Vidament* et al. (1997) reported fertility rates between 32 and 41% (total of 1.473 cycles, 1991-1995) when mares were inseminated two or more times per cycle with a total of either 150 x 10^6 or 300 x 10^6 frozen/thawed spermatozoa at 24 h intervals. From 1996 to 1999, a study using an increased AI dose of 400 x 10^6 frozen/thawed spermatozoa was carried out where the per cycle pregnancy rate was observed to be 49% (4190 cycles) (*Vidament* et al. 1999). The authors suggested that modifications in the technique for frozen/thawed semen in french National Studs (e.g. centrifugation and addition of freezing extender at 22°C; AI with 400 x 10^6 spermatozoa/AI every 24h before ovulation; more than one AI per cycle) were responsible for the improved fertility results being more equal to those from AI with fresh semen (56%, 2.050 cycles).

Minimal semen standard requirements for AI proposed by the World Breeding Federation of Sport Horses (WBFSH) are shown in Table 1. Fresh semen and chilled semen used within 12 hours after collection, should contain initially 300 x 10^6 pms with Δ 35% pms at the time of insemination. Chilled semen inseminated within 24 – 36 h after collection should have Δ 35% pms at the time of insemination and should initially be diluted to 600 x 10^6 pms. The minimum requirements for frozen/thawed semen post-thawing are 250 x 10^6 pms with Δ 35% pms. A breeding dose for insemination of one mare should contain a minimum of 3 insemination doses.

For frozen/-thawed semen, we found from our own studies that an insemination regime with a single insemination is sufficient when performed within 12 h before to 12 h after ovulation. For cooled semen the time interval for AI is a bit more brodened to 24 h before to 12 h after ovulation. "Re-insemination" 24 h later is recommended, if ovulation does not occur within the specified period after AI. The major beneficial effect of multiple inseminations per cycle on pregnancy rates seems to arise from the increased likelihood of AI within this optimal window but needs to be considered with the extra costs of semen or semen transportation (*Sieme* et al. 2003).

Tab 1 Minimum standard requirements for semen for AI published as proposals by the World Breeding Federation of Sport Horses (WBFSH)
Minimale Standardanforderungen für KB-Sperma veröffentlicht als Empfehlungen durch die World Breeding Federation of Sport Horses (WBFSH)

Minimum standard requirements for semen for A.I. (WBFSH)	
Fresh semen:	- minimum 300 million progressively motile sperm (pms)
Chilled semen:	
diluted/fresh sperm dose :	- minimum of 300 pms at time of portioning - insemination within 12 hours of collection - storage conditions maintained - pms at time of insemination not less than 35%
diluted/transported: sperm dose:	- minimum of 600 pms at time of portioning - maximum volume 40 cc (dilution 1:2) - insemination 24-36 hours after collection - storage conditions maintained - pms at time of insemination not less than 35% - all semen leaving the AI-center has to be treated as diluted/transport semen.
Frozen semen:	
volume dose: sperm dose:	- dependent on manufacturing process - minimum of 35% pms post-thawing - mininimum of 250 million pms per A.I. dose post-thawing - a breeding dose for insemination of one mare has to contain a minimum of 3 insemination doses. free to sell a single insemination dose

Site/Techniques of insemination

Site of sperm deposition

During natural mating and routine AI, semen is deposited directly into the uterine body. This is acceptable when sufficient good quality semen is available. However, if low numbers of spermatozoa are available alternative techniques may be desired. To study the effects of insemination technique on distribution of spermatozoa in the equine genital tract, 12 estrous mares were inseminated into the uterine body and 15 estrous mares into the uterine horn, ipsilateral to the preovulatory follicle with frozen/thawed spermatozoa (*Fêo* et al. 1992). One to two hours after AI mares were slaughtered and portions of the uteri were clamped in order to obtain seg-

ments of cervix, uterine body, uterine horns and oviducts. These segments were flushed and the number of spermatozoa in each was counted. Sperm numbers were significantly higher in the inseminated uterine horn as compared to the contralateral horn when intracornual inseminations was carried out. In addition, the number of spermatozoa in the inseminated uterine horn was significantly higher compared to both uterine horns when mares were inseminated in the uterine body. The authors concluded that this demonstrates the advantages of depositing semen closer to the site of ovulation.
Interstingly in the same study, the mean numbers of spermatozoa recovered in the right and left oviducts were not significantly different between uterine body and uterine horn inseminations. The site of uterine sperm deposition may not influence migration of spermatozoa into the oviduct.

In dairy cattle, site of semen deposition is discussed controversially. *Senger* et al. (1988) reported higher pregnancy rates for cornual inseminations compared to uterine body insemination, whereas *McKenna* et al. (1990) found no advantage using cornual insemination. *Hawk* et al. (1988) showed that insemination onto the infundibular surfaces of the cranial oviduct employing laparascopy reduced fertility significantly when compared to uterine body insemination (9% and 62% respectively). These findings suggested that exposure of spermatozoa to the uterus is important in achieving good fertility. In the same study semen was placed close to the UTJ after insertion of a flexible tube through the cervix. No difference was found in fertility when compared to uterine body insemination using either fresh or frozen/thawed semen. The authors suggested that in dairy cattle, the tendency for retrograde sperm transport is so strong that fertility is not higher even though semen is deposited much closer to the UTJ.

In equine AI, it remains open whether the site of semen deposition in the uterus affects migration of spermatozoa in the oviduct and the time required for a competent number of viable spermatozoa to move into the caudal isthmus has to been taken into consideration. The majority of spermatozoa are eliminated from the uterus within 4 h after AI (*Katila* 1995). *Scott* et al. (1995) reported a selection at the oviductal epithelium, favouring motile and morphological normal spermatozoa. They also found that sperm transport takes 4 hours in the mare. This implies, sperm transport may not have been complete in the study of *Fêo* et al. (1992). However, it is still unkown, how much time sperm transport really takes. It may be that only 15 to 30 minutes are required.

Insemination techniques

Sex-selection before fertilization is now possible by means of high speed flowcytometry, which seperates spermatozoa into X- and Y-chromosome bearing populations. However, limited number of spermatozoa being sorted per hour are insufficient for conventional insemination into the uterine body of mares. Therefore low dose insemination techniques were initially developed for the successful insemination of sex sorted spermatozoa (for review: *Lindsey* et al. 2001, *Morris* and *Allen* 2002). *McCue* et al. (2000) produced pregnancies by oviductal insemination with only 5 x 10^4 pms, but this method is impractical as it requires laparatomy. Deep uterine insemination has been reported employing a flexible catheter that is inserted in the uterine horn ipsilateral to the preovulatory follicle. The position of the catheter can be verified either by ultrasonography (*Buchanan* et al. 2000) or by transrectal palpation of the uterotubal papilla (*Woods* et al. 2000, *Rigby* et al. 2001). The hysteroscopic insemination - depositing semen onto the UTJ papilla using a videoendoscope - seems to produce acceptable pregnancy results with a small numbers of spermatozoa as previously reported by *Vasquez* et al. (1998), *Manning* et al. (1998), *Morris* et al. (2000).

Some literature report similar or even better results when semen was transfered to the uterine horn with the use of either a rectally guided insemination gun or by hysteroscopic insemination instead of routine insemination into the uterine body (*Féo* et al. (1992), *Woods* et al. (2000), *Morris* et al. (2000a), *Alvaraenga* and *Leào* 2002, *Petersen* et al. 2002). On the other hand *Manning* et al. (1998), *Buchanan* et al. (2000), *McCue* et al. (2000) and *Squires* et al. (2002) reported an opposite effect of the two insemination techniques on pregnancy rates of mares. These conflicting reports make it difficult to draw conclusions for AI practice and suggest that some essential factors varying between studies (e.g. sperm dose, sort of spermatozoa, technique of hysteroscopic insemination) have to be considered carefully (Tab. 2).

Intrauterine treatment with prostaglandin E2 prior to low dose (5 x 10^6 pms cooled/stored) transrectally-guided or hysteroscopic-guided deep intrauterine insemination in order to influence oviductal smooth muscle function and subsequent oviductal sperm migration has not been shown to result in increased pregnancies in the mare (*Brinsko* et al. 2003).

The use of low dose insemination techniques with frozen/thawed stallion semen however, may increase pregnancy rates. AI with frozen/thawed semen commonly produces lower conception rates as compared to AI with fresh or chilled stallion semen (*Pickett* et al. 2000). It is generally accepted that freezing causes semen damage and lowers the chances for pregnancy to spermatozoa (*Watson* 2001). Furthermore, the removal of seminal plasma related to the dilution of the ejaculates based on sperm numbers as well as centrifugation is disadvantageous. Studies suggest that seminal plasma helps to protect spermatozoa to facilitate optimal transport conditions in the genital tract (*Kotilainen* et al. 1994, *Troedsson* et al. 2002).

Deposition of spermatozoa close to the UTJ may increase the number of viable spermatozoa which reach the oviduct. In the literature the requirement of rectally or ultrasonographically guided intracornual or hysteroscopic techniques of low insemination dosages is another controversially discussed issue. In comparison to uterine body insemination, *Petersen* et al. (2002) observed no differences in embryo recovery rates when mares were inseminated twice (12h and 36h after hCG-administration) with only 50 x 10^6 pms frozen/thawed spermatozoa inseminated deep into the uterine horn, ipsilateral to the pre-ovulatory follicle in comparison to semen doses of 500 x 10^6 pms fresh semen inseminated into the uterine body 12h after hCG treatment and 500 x 10^6 pms cooled-stored spermatozoa 36h after hCG (7/11, 64% vs 4/11, 34%). As comapared to uterine body insemination, *Squires* et al. (2002) reported lower pregnancy rates when mares were

Tab 2 Overview: low dose insemination techniques in the mare *Übersicht zu Besamungstechniken mit geringer Spermienzahl bei der Stute*

Reference	AI technique	Type of sperm	Number of sperm (x 10^6)	Inseminate volume (ml)	Pregnancy rate	(%)
Feo et al. (1992)	ub	frozen	250 – 350	4	8/25	32
	uh				32/48	66
Manning et al. (1998)	ub	fresh	100 pms	<12	4/12	33
	ub		10 pms	2.5	2/12	17
	hs		10 pms	0.25	0/11	0
	hs		1 pms	<0.16	2/9	22
Vazquez et al. (1998)	hs	fresh	3.8 pms	0.2	3/10	33
Buchanan et al. (2000)	ub	fresh	500 pms	20	18/20	90
	uh		25 pms	1	12/21	57
	uh		5 pms	0.2-1	7/20	35
Woods et al. (2000)	ub	fresh	25	1	10/18	56
	uh				10/16	63
McCue et al. (2000)	ub	fresh	500 pms	20	6/15	40
	surg.		0.05 pms	0.05	3/14	21
Morris et al. (2000)	hs	fresh	10	0.03-0.15	6/10	60
			5		6/8	75
			1		16/25	64
			0.5		4/14	29
			0.1		?/11	22
			0.001		1/10	10
Morris et al. (2000b)	hs	frozen	25 pms	0.5	9/14	64
	ub				9/12	75
	hs		5 pms	0.1	16/34	47
	ub				2/8	25
Rigby et al. (2000)	uh	fresh	5	0.2	10/20	50
	hs				13/21	62
Alvarenga et al. (2001)	hs	frozen	100-150	2 x 0.5	12/22 centre A	54
					22/40 centre B	52
Alvarenga and Leao (2002)	hs	frozen	10 pms		4/12	33
	hs		10 pms	Percoll	4/12	33
	ub		400 pms		0/12	0
Petersen et al. (2000)	uh	frozen	50 pms	0.5 (2AI)	7/11	64
	ub	fresh	500 pms	(2AI)	4/11	37
Squires et al. (2002)	ub	frozen	800	2 (1 AI)	12/20	60
	ub		400	1 (2 AI)	11/20	55
	ub		200	0.5(2AI)	10/20	50
	uh		200	0.5(2AI))	4/20	20
Ismer (2002)	ub	stallion A	500 pms	10-20	49/86	57
	hs		6, 12, 80	0.2-1.6	32/108	29
	ub	stallion B	500 pms	10-20	214/305	70
	hs		100	5	9/20	45
	ub	stallion C	500 pms	10-20	3/8	37
	hs		100-200	10-20	37/68	55
Brinsko et al. (2003)	hs	cooled	5 pms	0.2 +PGE	6/9	67
	hs			0.2 +saline	6/9	67
	uh			0.2 +PGE	5/9	56
	uh			0.2 +saline	5/9	56
Morris et al. (2003)	ub	frozen	14 pms	0.5	8/12	67
	hs		14 pms	0.5	9/14	64
	hs		3 pms	0.1	16/34	47
	ub		3 pms	0.1	2/14	14
	hs		3 pms	0.1contral.	1/12	8

ub = routine insemination into the uterine body
uh = rectally controlled deep intrauterine insemination close to the utereotubal junction ipsilateral to the preovulatory folli cle
hs = hysteroscopic-guided deep intrauterine insemination onto the uterotubal junction ipsilateral to the preovulatory follicle

inseminated with only 200 x 10^6 frozen/thawed spermatozoa deep into the uterine horn, ipsilateral to the pre-ovulatory follicle (4/20, 20% vs 10/20, 50%). In contrast, *Morris* et al. (2000b) observed that hysteroscopic uterotubal insemination with 25 x 10^6 pms frozen/thawed spermatozoa hold no advantage over uterine body insemination (9/14, 64% vs 9/12, 75%). When the same authors used only 5 x 10^6 pms frozen/thawed spermatozoa, hysteroscopic insemination on the UTJ showed better results than when semen was deposited cranial to the cervix by means of a videoendoscope (16/34, 47% vs 2/8, 25%). In a further experiment *Morris* et al. (2003) observed significantly higher pregnancy rates when mares were inseminated with 3 x 10^6 pms frozen/thawed spermatozoa hysteroscopically compared to uterine body insemination (16/34, 47% vs 2/14, 14%). In the same study one of 12 mares became pregnant after hysteroscopic insemination of 3 x 10^6 pms frozen/thawed spermatozoa onto the UTJ contralateral to the preovulatory follicle, thus, retrograde sperm transport may play a role in the mare. *Alvarenga* and *Leào* (2002) found no significant difference between conception rates of mares inseminated with either 10 x 10^6 pms frozen/thawed spermatozoa hysteroscopically onto the UTJ, 10 x 10^6 pms of frozen/thawed and Percoll selected spermatozoa hysteroscopically onto the UTJ or 400 x 10^6 pms into the uterine body (4/12, 33.3%; 4/12, 33.3%; 0/12, 0%). The collective results from hysteroscopic insemination (both with and without Percoll selection) were significantly higher than after uterine body insemination. *Lindsey* et al. (2002a) observed lower pregnancy rates using 5 x 10^6 frozen/thawed sorted spermatozoa by hysteroscopic insemination, (2/15, 13.3%) as compared to frozen/thawed unsorted spermatozoa (6/16, 37.5%). Despite the use of low numbers of mares used in the described studies, the results indicate that deep intrauterine, low dose insemination of frozen/thawed spermatozoa may achieve reasonable pregnancy rates. *Alvarenga* et al. (2001) inseminated mares hysteroscopically within 6 hours on the side of ovulation with two 0.5 ml straws, each containing 50-75 x 10^6 spermatozoa with at least 30% postthaw motility at two different AI-centres. The pregnancy rates were 54.5% (12/22) and 52.5% (22/40) for center A and B respectively. Unfortunately in the latter study no control inseminations were reported.

The weighted mean of pregnancy rates of all the studies listed in Table 2 when frozen/thawed semen was transfered by either rectally controlled deep intrauterine insemination into the uterine horn ipsilateral to the preovulatory follicle (uh) or by hysteroscopic insemination (hs) were 54.4%; (43/79) and 50.5%; (92/182). Pregnancy rates after AI with fresh or cooled semen after uh insemination were 51.5% (49/95) and 41.9% (144/343) after hs insemination .
The concentration of semen has been shown to effect pregnancy rate as well. *Jasko* et al. (1992) reported a decrease in pregnancy rates when the sperm number of fresh stallion spermatozoa was below 25 x 10^6 pms. When frozen/-thawed semen was used, inseminate volume and total sperm number also effect pregnancy rate as shown in a multi-center study by *Samper* et al. (2002). Others produced pregnancies with low doses and small seminal plasma volumes employing hysteroscopic, uterotubal inseminations (*Morris* et al. 2000, *Alvarenga* et al. 2001), or rectally guided deep intracornual insemination (*Petersen* et al. 2002)

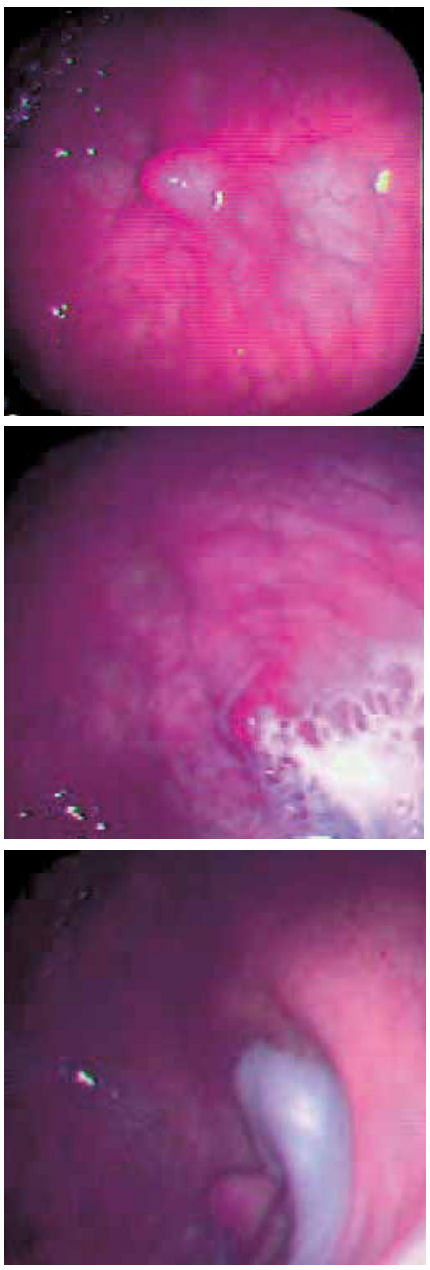
It seems questionable, whether such low sperm doses are really needed with respect to studfarm practice, bearing in mind that stallions produce approximately 5.0 to 7.0 x 10^9 spermatozoa per ejaculate (*Pickett* et al. 1987). As long as 50% of spermatozoa are progressively motile and using a standard dose of 50 x 10^6 pms cooled spermatozoa, 50 to 70 mares can be inseminated from a single ejaculate. This is plenty to cover the demand of semen from top stallions, possibly without the use of hysteroscopic or deep intauterine insemination techniques which require skilled experience of the inseminator. Consequently, the main application of deep intrauterine hysteroscopic insemination will for a foreseeable time be AI with sex sorted spermatozoa, either stored or frozen/thawed as reported by Lindsey et al. (2002 a,b,c).
Before extensive use of these different insemination techniques, some aspects of studfarm practice must have been taken into consideration. Firstly, payment policy, prices of

individual frozen/thawed semen doses and prices for veterinary services determine, which insemination method is economically the best. If the price is based on insemination doses with frozen/thawed semen, only one insemination per oestrus can often be afforded. Generally, a single insemination dose of frozen/thawed stallion spermatozoa is packaged in 4 to 8 x 0.5 ml straws (*Samper* et al. 2002). The frozen/thawed sperm dose recommended in many reports (*Leipold* et al. 1998, *Vidament* et al. 1999, *Samper* et al. 2002) could obviously been reduced without risking a decrease in fertility as demonstrated in the studies of *Morris* et al. (2001), *Alvarenga* et al. (2001) and *Petersen* et al. (2002).

Another aspect of hysteroscopic insemination using semen from stallions with low fertility is currently discussed contraversial. *Morris* and *Allen* (2002) summarized that use of hysteroscopic insemination with low numbers of spermatozoa from infertile stallions does not improve their fertility. In contrast, *Koene* et al. (2002) and *Ismer* (2002) observed a significant increase in pregnancy rates when mares were inseminated hysteroscopically (37/68, 55.2%) instead of with conventional AI (3/8, 37.5%) into the uterine body with semen from one particular stallion. The stallion showed asthenozoospermy and a history of reduced fertility. However, in the same study the authors reported a significant reduction in pregnancy rates from hysterscopic insemination vs uterine body AI when semen of two normospermic stallions was used (stallion A: 32/108, 29.6% vs 49/86, 57.0%; stallion B: 9/20, 45.0% vs 204/305, 70.2%). Further studies are required to discover which specific sperm defects may be compensated by different insemination sites/techniques.

Furthermore, it is evident that inseminations in mares are followed by post breeding endometritis (for review: *Katila* 2001, *Troedsson* et al. 2001). Therefore one may suggest the use of of lower numbers and volumes of sperm directly on the uterotubal papilla reduce risk of post breeding endometrits (*Lindsey* et al. 2001). However, *Schiemann* et al. (2001) detected in 4 out of 8 healthy diestrous mares pathogenic microbes (3 x Pseudomonas aeruginosa, 1 x b-hem. Streptococcus), five days after diagnostic hysteroscopy. Six mares showed inflammatory endometrial reaction and a remarkable eosinophilia as detected in histological preparations of endometrial biopsies. The same authors recommend a follow up treatment for mares undergoing hysteroscopy and speculated that etiology of eosinophilia was induced by distension of the uterus with air. Further studies are required to determine hygienic risks of hysteroscopic inseminations in problematic mares, as previously suggested by *Squires* et al. (2002).
During the breeding season 2002, our group investigated the effect of different insemination techniques on twin pregnancy rates in mares that had double-ovulation either ipsilateral or contaralateral. Hanoverian warmblood mares were inseminated once per cycle, 24 h after hCG administration with cooled semen, and alternatively 30 h after hCG with frozen/thawed semen. Mares were placed randomly into groups, each inseminated with a different technique [i.e., routine insemination into the uterine body (ub) and deep into the uterine horn (uh) ipsilateral to the preovulatory follicle the latter either rectally controlled or hysteroscopically]. Differences in fertility results between treatment groups were analysed by Chi-squared test using the SAS statistics package. Images of the UTJ of some of the mares inseminated with the help of a videoendoscope are presented in Figure 1. Out of the 331 mares, double ovulation was detected in 35 (10.6%) 19 ipsilateral and 16 contralateral. Pregnancy rates were similar after ub or uh insemination insemination. Twin pregnancy rates were not different in mares inseminated into the uterine body after either ipsilateral or contralateral double ovulation; whereas after deep intrauterine insemination into the uterine horn twin pregnancy rate were significantly reduced when double ovulations occured contralateral (Table 3).

Fig 1 Hysteroscopic view onto the uterotubal junction showing the uterine papilla ipsilateral to the preovulatory follicle of an estrous mare (a). Presence of bubbles after deposition of low number of sperm suspended in a small inseminate volume (b). Distribution and reflux of semen after hysteroscopic insemination of a commercial inseminate volume (~12 ml) (c)
Hysteroskopische Darstellung der Uterotubalpapille ipsilateral zum präovulatorischen Follikel einer östrischen Stute (a). Adhäsion eines geringvolumigen Inseminats an der Uterotubalpapille mit Bläschenbildung (b). Verteilung und Reflux des Inseminats nach hysteroskopischer Insemination eines kommerziell üblichen Inseminationsvolumens (~12 ml) (c)



Tab. 3 Twin pregnancy rates after routine insemination into the uterine body (ub) and inseminations deep into the uterine horn (uh, either rectally controlled or hysteroscopically) with respect to side of double ovulation (ipsilateral, contralateral) in warmblood mares.
Zwillingsträchtigkeitsraten nach Routine-Samenübertragung in den Gebärmutterkörper (ub) und tiefintracornualer Besamung (uh, entweder rektal geleitet oder hysteroskopisch) unter Berücksichtigung ipsi- oder contralateraler Doppelovulationen bei Warmblutstuten.

Group	Mares		Pregnancy ipsilateral		Pregnancy contralateral		Twins ipsilateral		Twins contralateral	
	(m/n)	%	(m/n)	%	(m/n)	%	(m/n)	%	(m/n)	%
Σ ub	71/139	51.1	5/10	50.0	4/6	66.6	2/10	20	2/6	33.3
Σ uh	101/192	52.6	5/9	55.5	7/10	70.0	4/9	44.4[1]	0/10	0[2]
Σ ub + Σ uh	172/331	52	10/19	52.6	11/16	68.7	6/19	31.6	2/16	12.5

(m/n) = number of pregnant mares (m) out of total number of mares inseminated (n)
% = percentage of pregnant mares
ub = routine insemination into the uterine body
uh = deep insemination into the uterine horn (either rectally controlled or hysteroscopically)
pregnancy ipsilateral = pregnancy rates in mares with ipsilateral double ovulations
pregnancy contralateral = pregnancy rates in mares with contralateral double ovulations
twins ipsilateral = twin-pregnancy rates in mares with ipsilateral double ovulations
twins contralateral = twin-pregnancy rates in mares with ipsilateral double ovulations
1:2 $p < 0,05$

These data suggest that the incidence of twin pregnancy rates after contralateral double ovulations could possibly be reduced by the technique of insemination. Nevertheless, the authors do not recommend deep intrauterine insemination to reduce incidence of twin pregnancies in general for studfarm practice, especially as double ovulations increase the chances of pregnancy. The method of choice in managing equine twins in the mare is still early transrectal ultrasonographic detection of twins and manual crush of one embryonic vesicle as previously reported by *Macpherson* and *Reimer* (2000).

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