Breeding with frozen semen: what are the considerations?

The use of frozen semen is sometimes the only available option for artificial insemination. Compared to fresh or chilled semen, the use of frozen semen has previously been reported to have lower pregnancy rates, and higher rates of post-breeding inflammation and uterine fluid accumulation. More recent studies have found that pregnancy rates are indeed lower than with fresh semen, but are comparable if not better than chilled semen, with little evidence of increased complications. Several factors can affect conception rates and the practicality of using frozen semen, and these limitations should be explained to the client in advance. This review covers essential requirements applicable to artificial insemination with frozen semen, as well as mare and stallion factors that contribute to the adaptation of appropriate insemination protocol.

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Insemination with fresh semen has a per-cycle pregnancy rate of up to 76% (Jasko et al, 1992), and could therefore be regarded as the gold standard. However, vets are often faced with less-than-ideal conditions and chilled and frozen semen are often used instead.

The evidence on per-cycle pregnancy rates for chilled and frozen semen are conflicting (*Table 1*). Some studies have reported higher per-cycle pregnancy rates for chilled semen (Jasko et al, 1992; Loomis, 2001), while others reported higher pregnancy rates per-cycle, as well as higher seasonal pregnancy rate, in mares artificially inseminated with frozen semen (82% frozen, 69.6% chilled, p=0.02; Crowe et al, 2008). Before considering the factors that may affect pregnancy rates, the following areas should be considered as these could potentially eliminate frozen semen as an option or have a large impact on the insemination protocol used.

Essential requirements

Equipment/expertise

The use of frozen semen for artificial insemination requires specialised equipment, such as liquid nitrogen storage tanks, heated water baths and microscopes with a heated stage. Appropriate expertise is equally important, athough this is more difficult to quantify. The British Equine Veterinary Association (BEVA) sets out requirements for continuing professional development in order to join the BEVA approved artifical insemination list (Griffiths, 2021).

As for minimum number of cases, based on Samper et al (2008), it seems sensible to aim for a caseload of at least 15 artificial inseminations a year, since their reported pregnancy rates were 10% higher for practices inseminating more than 15 mares.

Documentation

No semen should be inseminated without accompanying documentation clearly identifying the consignment and confirming the stallion's health status, as outlined in the Horserace Betting and Levy Board International Codes of Practice (2022). Electronic copies of original documentation are acceptable, provided they are accompanied by paper copies of the originals within a few days (Griffiths, 2021).

Logistics

The client should be able to transport their mare to the clinic where equipment, staff and semen storage are available and the premises are compliant with health and safety regulations. Liquid nitrogen should never be transported in the car because of the risk of spillage, which could cause serious physical harm to humans and the vehicle by resulting in asphyxiation of the passengers by depletion of oxygen (NHS Fife, 2019). Only cars fitted with special equipment and drivers with extra training in transporting hazardous substances should transport liquid nitrogen.

Factors affecting conception rates

Mare factors

Mare age, status and susceptibility to post-breeding complications have been studied extensively, but the results across different studies have often been conflicting.

Increased age has been associated with lower pregnancy rates with frozen semen (Barbacini et al, 1999). However, further studies have suggested that increasing age did not affect frozen semen pregnancy rates but, interestingly, did reduce chilled and fresh semen pregnancy rates (Squires et al, 2006; Lewis et al, 2015). As for

Table 1. Pregnancy rates for fresh, chilled and frozen semen artificial insemination.

Study			Results (per cycle)		
Year (chronological)	Authors	Study details	Fresh semen	Chilled semen	Frozen semen
2001	Loomis, 2001	850 mares, 16 stallions (chilled) 876 mares, 106 stallions (frozen)	n/a	59%	51%
2006	Squires et al, 2006	961 mares, multicentric	60%	44%	46%
2010	Crowe et al, 2008	251 mares	n/a	44%	59%
2015	Lewis et al, 2015	578 mares, 240 stallions, 3 years	63%	43%	48.6%

mare status, there seems to be a consensus that older maiden mares have lower pregnancy rates after artificial insemination with frozen semen (Barbacini, 1999; Squires et al, 2006; Lewis et al, 2015), although this reduction appears no greater than that for chilled semen (Lewis et al, 2015). Conception rates during foal heat are lower (Lieux, 1980; Loy, 1980) and this should be explained to the owner. Unless time pressure is a factor, insemination during foal heat should be avoided if maximal conception rates are to be achieved.

'Problem' mares and complications

There have been concerns that frozen semen is associated with increased problems, such as accumulation of uterine fluid and persistent-breeding induced endometritis (PBIE). However, from the research it appears that these problems occur predominantly in mares susceptible to PBIE and among healthy mares, the risk of complications using frozen semen is negligible.

Several studies reported that frozen semen is not associated with increased breeding-induced inflammation or uterine fluid accumulation when compared to other insemination types (Squires et al, 2006; Lewis et al, 2015; Newcombe and Kelly, 2016), and may induce even less inflammation or fluid. In mares susceptible to delayed uterine clearance and PBIE, the pregnancy rate by any method is reduced. The presence of periovulatory uterine fluid has been associated with lower pregnancy rates (Pycock and Newcombe, 1996) and the decrease in pregnancy rates was similar for both frozen and chilled semen (Lewis et al, 2015).

Even the use of multiple insemination doses does not seem to increase the risk of complications (Reger et al, 2003; Squires et al, 2006). Huber et al (2019) inseminated four quarter-doses at regular periovulatory time intervals and found that this did not lead to any increase in fluid or inflammation. It would therefore appear that the amount of semen (chemical effect) or frequency of insemination (mechanical effect) has minimal or no effect on the risk of development of PBIE.

Unsurprisingly, the use of frozen semen in 'problem' mares is avoided by many practitioners in view of generally lower conception rates (Jasko et al, 1992; Sieme et al, 2004). Similarly, the use of hysteroscopic artificial insemination may be discouraged in these mares as there is an increased risk of uterine inflammation, but even in this aspect the literature is not unified in opinions (Köhne et al, 2020). Ferrer et al (2012) found a transient inflammatory response which increases with the procedure duration, but also found that even in mares susceptible to PBIE the response was apparent at 24 hours but not at 48 hours, thus concluded that there was no contraindication for its use in these mares.

Stallion and semen factors

Stallion factors can be divided into the semen quality and quantity (number of straws). Both of them are closely related to the insemination protocols, which can compensate to the degrees of poorer quality or low quantities of semen.

Quality

The semen should be assessed as being sufficient quality before freezing, but even this does not guarantee a good quality post-thawing. Post-thaw motility and fertility rates vary between stallions (Amann and Pickett 1987) and keeping a proven record of a stallions' pregnancy rates with frozen semen would give the clinician more certainty of expected pregnancy rates, but this is not always available. It is generally accepted that a post-thaw insemination dose should contain 200-250 million progressive motile sperm with a minimum post-thaw motility of 30%.

Quantity

Variability also exists in the number of straws and total insemination dose, which contribute to the achievement of acceptable pregnancy rates. There appears to be an individual stallion-dependent threshold, which unless exceeded, leads to poor pregnancy rates (Samper et al, 2008). This threshold is often unknown and without knowledge of the stallions' pregnancy rate for a particular insemination dose or number of straws, it is very difficult to give the client a realistic likelihood of conception in the mare. This is important as in clinical practice, clients may request use of 1 straw/0.5ml frozen insemination dose for cost reasons. Using low dose insemination may result in low conception rates and can therefore be a false economy, which should be discussed with the client.

Veterinary management

There are various insemination protocols with similar pregnancy rates, which can be adopted based on semen availability (1 straw versus multiple doses), staff availability and equipment. For simplicity, protocols can be divided into three categories but in the clinical practice, there are many variants based on the clinician's preference (*Table 2*).

Once the follicle reaches adequate size (usually 30–40mm) an ovulation induction agent is administered. Fixed-time protocols

Table 2. Insemination protocols

Insemination protocol	General description	Advantages	Disadvantages	Reported pregnancy rates
Fixed-time single insemination	Adequate follicle size Ovulation agent administered Artificial insemination once at fixed interval (no ultrasound monitoring)	Less ultrasound examinations One insemination dose One insemination (thawing, preparations, staff usage)	Semen wasted if abnormal or non-ovulation occurs	54.7% (Hollinshead and Hanlon, 2018)
Fixed-time double insemination	Adequate follicle size Ovulation agent administered Artificial insemination twice at fixed intervals (no or minimal ultrasound monitoring)	Less ultrasound examinations	Bigger/double semen dose or single dose splitting Two inseminations (thawing, preparations, staff usage) Semen wasted if abnormal or non-ovulation occurs (some clinicians wait for ovulation to inseminate second dose)	59% (Crowe et al, 2008) 76.4% (Reger et al, 2003)
Continuous interval monitoring	Adequate follicle size +/- Ovulatory agent administered Examination intervals 6–12 hours over expected periovulatory period Artificial insemination 12 hours pre-/post-ovulation depending on clinician's preference	One insemination One insemination dose If abnormal or non-ovulation occurs – semen is conserved Artificial insemination at preferred time (pre- or post-ovulation)	Multiple ultrasound examinations	48.6% (Lewis et al, 2015)

Please note only general description is given, these protocols vary between reports, for specific details please refer to studies referenced.

rely on insemination being close to the time of this induced ovulation (usually 6–12 hours). This is performed with no or minimal ultrasound examinations in between. In continuous interval monitoring, the timing of artificial insemination is determined based on repeated ultrasound examinations.

Timing of insemination

Maximal pregnancy rates with frozen semen have been reported when insemination occurs within 12 hours pre-ovulation and 6–12 hour post-ovulation with comparable results (Barbicini et al, 1999; Sieme et al, 2003; Squires et al, 2006). Many clinicians choose to examine mares at 6-hour intervals, although pregnancy rates and embryo loss rates are not reduced when monitoring intervals increase to 12–15 hours (Newcombe et al, 2011; Immonen and Cuervo-Arango, 2020).

When only one dose is available, post-ovulation insemination is preferred by many practitioners to ensure that semen is deposited only when a normal ovulation has occurred. Timing of insemination (pre- or post-ovulation) does not affect the risk of uterine fluid accumulation and embryo loss (Barbacini et al, 1999; Watson et al, 2001).

Number of inseminations

Multiple inseminations (2–3 doses) seem to increase the conception rate with chilled semen, but not as consistently with frozen semen (Squires et al, 2006). However, a study by Huber et al (2019) on frozen artificial insemination suggested that dividing one dose into four insemination quarter-doses lead to increased pregnancy rates when compared to two half-doses (73% versus 50%). This suggests an advantageous effect of multiple inseminations, rather than the total amount of semen used. This substantial increase in pregnancy rate, if repeatable and consistent, may encourage clinicians to consider this strategy, particularly when only one insemination dose is available. However, the process of thawing and inseminating one dose in four portions is very time and labour consuming.

Site and technique of insemination

Semen can be deposited through the cervix blindly into the uterine body, horn (deep uterine insemination) or hysteroscopically onto the oviductal papilla under direct vision (hysteroscopic insemination). Uterine body and deep uterine insemination require less staff and equipment and are sufficient in most clinical situations.

For frozen insemination with a standard dose, the difference among these methods is negligible (Morris et al, 2003; Samper et al, 2008). When only a small dose of frozen semen is available, hysteroscopic insemination and deep uterine insemination are the methods of choice and hysteroscopic insemination is the preferred method when total semen dose is less than 10 million progressively motile sperm (Morris et al, 2003; Govaere et al, 2014). EQ

Conflicts of interest

The author declares that there are no conflicts of interest.

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KEY POINTS

- Frozen semen conception rates are comparable to chilled semen but lower than fresh semen.
- Appropriate equipment, expertise and documentation must be available.
- Mare factors are not as important as stallion factors.
- Available semen dose and previous frozen artificial insemination record are key in estimating success rate and choice of insemination protocol.
- Use of single straw insemination is advisable only in stallion with proven frozen artificial insemination record using this dose.
- Deep uterine insemination is adequate in most situations, whereas hysteroscopically-guided insemination is recommended if total semen dose <10 million progressively motile sperm.
- The client should be made aware of realistic pregnancy rates and any increased costs in advance of the change to using frozen semen.

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