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Original Research

The Effect of Storage Temperature of Stallion Semen on Pregnancy Rates

Juan Cuervo-Arango [a,*], Kaisa Nivola [b], Leena Väihkönen [c], Terttu Katila [d]

[a] Departamento Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad CEU Cardenal Herrera, Alfara del Patriarca, Spain
[b] Kallelan Oriasema, Kalanti, Finland
[c] Hevoskylän klinikka, Orimattila, Finland
[d] Department of Production Animal Medicine, University of Helsinki, Saarentaus, Finland

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ABSTRACT

A new cooling method to store and transport semen in Styrofoam boxes at a temperature of 15°C to 20°C was developed. The new system was compared with the standard procedure in a field trial using pregnancy rate (PR) of inseminated mares as the benchmark. The standard transport method (F) involved the use of an overnight frozen cooling device, which resulted in a temperature range of 1°C to 8°C inside the syringe containing the semen. The test method (R) involved a cooling device kept in the refrigerator overnight to achieve temperatures inside the semen syringe of 15°C to 20°C. A total of 409 ejaculates from 15 stallions housed at two stud farms were cooled and then transported to inseminate 195 mares one to three times per estrus during one to four cycles in the same breeding season. Altogether, 255 inseminations were carried out using semen cooled by the F method and 154 cooled by the R method. The PRs were not significantly different between the two cooling methods (per cycle PR in the F group = 45.0% vs. 44.6% in the R group). In experiment 2, 13 mares were inseminated in 25 cycles using F semen (n = 12) and R semen (n = 13) stored for 12 hours. The mares were administered hCG 12 hours after artificial insemination (AI) and scanned every 12 hours to determine the time of ovulation. The semen temperature had no effect on PR. All mares that ovulated within 12 hours of AI (4 of 4) conceived. However, only two of 21 mares (9.5%) that ovulated >36 hours after AI conceived.

1. Introduction

Insemination of mares with cooled transported semen is commonly practiced in horse breeding worldwide. Several different types of containers are used to transport the semen among which disposable, inexpensive, Styrofoam-type boxes have gained popularity in recent years [1]. The standard procedure is to cool the extended semen slowly and aim for a final temperature of 4°C to 10°C [1].

The metabolism of spermatozoa at body temperature is very high and produces a lot of waste products such as lactic acid and oxygen free radicals. Lactic acid increases acidity, which decreases enzyme activity, which in turn results in a loss of adenosine triphosphate production. Oxygen free radicals induce lipid peroxidation, which causes membrane permeability and enzyme dysfunction, leading to a loss of cell integrity, motility, and viability [2]. With a reduction from body temperature to 4°C, metabolism decreases to only 7% of that at body temperature. Lower temperatures between 0°C and 2°C are detrimental to sperm viability, whereas temperatures above 10°C may not reduce sperm metabolism sufficiently [2].

Not only the temperature per se, but also the cooling rate of semen during the phase transition of the sperm

* Corresponding author at: Juan Cuervo-Arango, Departamento Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad CEU Cardenal Herrera, 46115 Alfara del Patriarca, Spain.
E-mail address: juan.cuervo@uch.ceu.es (J. Cuervo-Arango).

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membrane from a fluid to a gel state (i.e., between 9°C and 19°C) is relevant to sperm viability. Ideally, this should be around −0.05°C/min [2,3]. Cooling and storage of semen would not be possible without extenders. They minimize microbial growth, provide nutrients and a proper balance of mineral elements, protect against cold shock, stabilize enzyme systems and membrane integrity, and neutralize toxic products [4].

Semen from most stallions survives the standard procedure of cooling to 4°C and retains a good level of fertility for 48 to 72 hours if maintained at this temperature [1,2,5]. However, the cooling process stresses and may damage spermatozoa resulting in motility changes and senescence before artificial insemination (AI) [6], which then reduces the longevity of the spermatozoa in the mare's reproductive tract after they have warmed to body temperature. Decreased pregnancy rates (PRs) have been reported when the interval from AI with cooled stored or transported semen to ovulation is ≥24 hours [7,8]. On the other hand, fresh spermatozoa deposited in the mare's genital tract by natural mating or AI can remain viable for a much longer period than chilled semen, typically three to four days [9,10]. In a clinical setting, the time from AI to ovulation frequently exceeds 24 hours, especially if the mares to be inseminated are not treated with an ovulation-inducing hormone the day before the expected insemination. In some countries, reinsemination after 48 hours if the mare has not ovulated is commonly practiced. However, this protocol is time consuming and expensive, and it may cause problems in mares with delayed uterine clearance.

An alternative approach to reduce the negative impact of low temperature during semen storage would be to maintain the spermatozoa within a temperature range low enough to decrease metabolism before insemination but not so low as to damage their longevity in the mares' reproductive tract. A number of authors have shown that it might be beneficial to store semen at higher temperatures of 15°C to 20°C (Table 1) [11–15]. Many experiments have evaluated sperm characteristics after cooling and storage, but most did not examine the fertility component. Although total sperm motility was lower after storage at 20°C than immediately after collection of the ejaculate or after its storage at 5°C, day 15 PRs were not different [13] (Table 1). Batellier et al [14] reported significantly greater PRs in mares inseminated with semen stored at 15°C compared with those inseminated with semen kept at 4°C. Furthermore, insemination with semen stored at 15°C for 72 hours yielded acceptable PRs of 48% compared with a 68% conception rate in mares inseminated immediately after semen collection [14] (Table 1). However, in the studies by Batellier, the interval from AI to ovulation was unknown, so that the effect of semen storage temperature on sperm lifespan within the mares' reproductive tract could not be determined.

Table 1
Publications that have shown stallion semen storage temperatures of 15°C to 20°C to be better than lower storage temperature.

Reference	Temperature and Storage Time	Air/Extender	Dilution, Centrifugation, Concentration, and Sperm Numbers	Motility	Fertility, n	Differences
Province et al [11]	20°C and 12 hr; 15°C and 12 hr; 10°C and 12 hr; 5°C and 12 hr	Anaerobic, SM (heated skim milk), CAP (caprogen), or NFDMS (nonfat-dried milk solids)—glucose extender		SM 20, CAP 37, and NFDMS 18%; SM 14, CAP 20, and NFDMS 15%; SM 8, CAP 12, and NFDMS 11%; SM 5, CAP 8, and NFDMS 9% (PM)	6 stallions	20°C and 15°C better than 10°C or 5°C with all extenders
Francl et al [12]; Exp. 1; Exp. 2	20°C and 12 hr; 20°C and 24 hr	Anaerobic, EZ-Mixin; Anaerobic, EZ-Mixin	250×10^6 PMS; 250×10^6 PMS	34%; 17% (TM)	ERR 50%; ERR 62%	No difference between 37°C and 1 hr; No difference between 37°C and 1 hr
Varner et al [13]	Fresh and 0.5 hr; 20°C and 24 hr; 5°C and 24 hr	Anaerobic, NFDMS-Glucose extender	250×10^6 PMS; 250×10^6 PMS; 250×10^6 PMS; conc. 25×10^6/ml	89%; 57%[a]; 80% (TM)	73%, n = 15; 73%, n = 15; 73%, n = 15; day 15 PR (day 15 PR)	* Significantly different from the others
Batellier et al [14]; Exp. 1; Exp. 2	15°C and 24 hr; 4°C and 24 hr; Fresh and 0 hr; 15°C and 72 hr	Aerobic, INRA96; Anaerobic, INRA82 or Kenney extender; Aerobic, INRA96	conc. 20×10^6/mL; 200×10^6 TS; conc. 20×10^6/mL; 200×10^6 TS		57%[a], n = 178; 40%, n = 173; day 14 PR; 68%; n = 52; 48%; n = 52[a] (day 14 PR)	* Significantly different from 4°C * Significantly different from 15°C
Vidament et al [15]	5°C and 72 hr; 15°C and 72 hr	Aerobic, INRA96; Anaerobic, INRA96; Aerobic, INRA96	conc. 20×10^6/mL; 200×10^6 TS	29%; 23% (PM)	60%; n = 40; 60%; n = 40	

Abbreviations: ERR, embryo recovery rate; PM, progressive motility; PMS, progressively motile sperm; PR, pregnancy rate; TM, total motility; TS, total number of sperm.
[a] Significantly different ($P < .05$).