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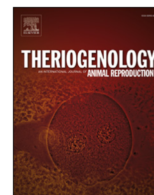
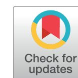


Association of equine sperm population parameters with outcome of intracytoplasmic sperm injections



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ABSTRACT

Limited clinical information is available regarding sperm population parameters that are important for use with equine intracytoplasmic sperm injection (ICSI). Therefore, the appropriateness of a sample of sperm is typically not known before ICSI. The aim of our study was to determine which sperm population characteristics were predictive of ICSI outcome. Frozen-thawed sperm samples (n = 114) from 37 stallions in a clinical program were analyzed after ICSI for percentages of normal morphology (MORPH+), live as assessed by eosin/nigrosin stain (LIVE+), membrane intact as assessed by hypoosmotic swelling test (HOS+), and DNA fragmentation determined by sperm chromatin dispersion (DNA−). ICSI was performed on 147 oocytes, and cleavage (≥2 cells), embryo development (morula or blastocyst), and pregnancy status after embryo transfer were determined. Among the examined sperm parameters, LIVE + correlated positively with MORPH+ and HOS+, and MORPH + negatively with DNA−; no other significant correlations were observed. When used for ICSI, sperm population percentages for MORPH+ and DNA− were not predictive of ICSI outcome, including cleavage, embryo development, and establishment of a pregnancy. Sperm population percentages significantly affecting ICSI outcomes were LIVE+ and HOS + for oocyte cleavage, LIVE + for embryo development, and HOS + for establishment of a pregnancy. The probability of a pregnancy was significantly higher for sperm populations having HOS+ ≥40% than populations having HOS+ ≤20%. The mean age of the donor mare per sperm-injected oocyte did not differ for oocyte cleavage, embryo production, or establishment of pregnancy. In our study, the probability of sperm-injected oocytes to develop into an embryo (morula or blastocyst) improved when sperm were selected from a population with higher indicators of membrane integrity (LIVE+ and HOS+).

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1. Introduction

Intracytoplasmic sperm injection (ICSI) has predominantly been used in human reproductive medicine. In the last 1.5 decades, ICSI has been developed for use in the equine industry for a diversity of reasons – including fertility status, gamete availability, and scheduling around performance activities. Frozen-thawed sperm are frequently used for equine ICSI [1]. Cryopreservation reduces sperm viability, motility, and fertility, although the detrimental effects of cryopreservation on sperm quality vary widely among stallions [2]. In men, sperm fertilizing potential and subsequent embryo development and pregnancy establishment are positively associated with normal sperm morphology, motility parameters, and DNA integrity [3,4]. Minimal information is available on the relationship with the population parameters of normal sperm morphology and ICSI outcome in horses. The final step of sperm selection for equine and human ICSI is often performed under X 200 or 400 magnification and based on gross morphology and motility [5–7]. However, these two sperm characteristics may be insufficient to select an ideal sperm, especially if morphological and functional changes have occurred after cryopreservation. Consequently, use of a compromised sperm for ICSI can result in failure of fertilization or embryo development in horses [8], and it has been associated with an increased abortion rate in women [9].

Associations of sperm population quality parameters with the success of ICSI are limited in horses, although they have been more thoroughly investigated in other species. Selection of human sperm by morphology and motility does not exclude sperm with DNA damage from being selected for ICSI, primarily for men with male factor infertility [10]. A higher percentage of sperm with normal morphology has damaged DNA in infertile when compared to fertile men [11], which negatively impacts embryo quality and the

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probability of pregnancy after ICSI [12]. In cattle, sperm population with more morphologically abnormal sperm are negatively associated with oocyte cleavage, embryo development, and blastocyst formation after IVF [13]. Sperm DNA fragmentation has also been associated with reduced fertility. In men, sperm DNA fragmentation has a detrimental effect on conception in vivo and pregnancy outcomes after fertilization in vitro [4,14], and it is negatively associated with sperm concentration, motility, and normal morphology [15,16]. The sperm's plasma membrane has essential functions for fertilization in vivo; however, characteristics of the plasma membrane also appear to be important for assisted fertilization. Sperm that display a functional plasma membrane under hypoosmotic conditions have lower incidences of abnormal head morphology, apoptotic markers, protamine deficiency, membrane damage, and DNA fragmentation, all of which relate to fertility potential [17–20]. Pregnancies and live births have resulted from nonmotile, human sperm populations when individual sperm are selected for ICSI based on membrane swelling under hypoosmotic conditions [21–25], suggesting that hypoosmotic swelling of the sperm membrane is indicative of factors beyond those required for motility. The associations between sperm characteristics and clinical success in other species suggest that sperm population parameters could be valuable considerations for equine ICSI. Although limited clinical information is available regarding which sperm factors affect equine ICSI outcome [26], the individual stallion does affect cleavage and embryo development rates [8,27,28]. Stallions with low or no field fertility have lower cleavage and blastocysts rates after ICSI [26,29,30]. The association between specific sperm characteristics and equine ICSI outcome has not been adequately studied.

The aim of our study was to determine sperm population parameters associated with equine ICSI outcome. To do so, we first identified assays to be used with the low numbers of sperm that were available after ICSI. The first objective was to determine correlations among different assessment parameters in samples from which sperm were selected for clinical ICSI. Sperm remaining after the ICSI procedure was completed were assessed for percentages of: 1) normal morphology, 2) live sperm, 3) membrane integrity based on hypoosmotic swelling, and 4) DNA fragmentation. Our second objective was to relate sperm population parameters from which a sperm was selected for ICSI to: 1) cleavage, 2) development of a morula or blastocyst acceptable for embryo transfer, and 3) establishment of early pregnancy.

2. Materials and methods

2.1. Chemicals and reagents

Unless noted, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sperm samples and oocyte injections

Frozen-thawed, sperm samples (n = 114 from 37 stallions) were obtained after sperm processing for clinical ICSI procedures. Sperm samples were analyzed for the following parameters: 1) percentage of sperm with normal morphology, MORPH+, 2) percentage of live sperm, as a measurement of viability and sperm head membrane integrity, LIVE+, 3) percentage of sperm with positive hypoosmotic swelling, indicative of intact principal piece membrane, HOS+, and 4) percentage of sperm with chromatin dispersion, consistent with fragmentation of DNA, DNA-. Sperm were obtained for analyses from samples processed by swim-up or direct dilution procedure for ICSI at approximately 5 min after ICSI was performed.

Frozen-thawed sperm samples were obtained from partial sections of straws (0.25, 0.5 or 5 mL) that were cut under liquid nitrogen. The swim-up procedure was used when frozen sperm had sufficient motility and sperm numbers to complete the procedure. For swim-up, the cut section of straw was placed directly into 1 mL of G-IVF (G-IVF™, Vitrolife Sweden AB, V, Frölunda, Sweden; with the addition of 0.4% BSA) at 38.2 °C, and the tube was placed at an approximately 45° angle in an atmosphere of 6% CO_2 and air for 15 min at 38.2 °C. The supernatant (500 μL) was removed and placed into 2 ml of gas equilibrated G-IVF and centrifuged for 5 min at 308 × g. The supernatant was removed, and sperm from the pellet was selected for ICSI. Frozen-thawed samples with poor quality or limited sperm motility were used for ICSI after direct dilution. Sperm were thawed by placing a section of cut straw into 0.25–1 mL of a commercial bench-top medium, G-MOPS™ (Vitrolife Sweden AB) with 0.4% BSA at 38.2 °C, and a sperm from the diluted sample was selected for ICSI. Sperm for oocyte injections were selected by a single technician based on microscopic evaluation for motility and normal morphology at X 200 magnification.

Oocyte donors were light-horse mares, 7 to 26 y (n = 42, mean ± SEM of 17.6 ± 0.50 y), in a clinical ART program. Mare reproductive tracts were examined using transrectal ultrasonography. When a growing follicle(s) with a mean diameter of approximately 35 mm and uterine edema was observed during the follicular phase, an ovulation inducing compound (human chorionic gonadotropin and/or deslorelin acetate) was administered to induce follicle maturation. The following day, between 18 and 24 h after maturation induction, oocytes were collected by ultrasound-guided, transvaginal aspirations. Oocyte collection attempts were made from the dominant follicle to obtain maturing oocytes, probably in metaphase I; in some mares, one to three of the larger subordinate follicle(s) were aspirated to obtain immature oocytes, probably in the germinal vesicle stage. Maturing oocytes from dominant follicles completed maturation in Medium 199 with Earle's salts, L-glutamine, and 2.2 g/L sodium bicarbonate (GIBCO BRL Life Technologies, Grand Island, NY, USA; with additions of 10% fetal calf serum, 0.2 mM pyruvate and 25 mg/mL gentamicin) for approximately 20 h at 38 °C and in 6% CO_2 and air. Oocytes from subordinate follicles were cultured under the same conditions in Medium 199 with the added supplementation of hormones and growth factors [15 ng/ml FSH (National Hormone & Peptide Program, NHPP, Torrance, CA USA), 1 μg/ml LH (NHPP), 50 ng/ml EGF, 100 ng/ml IGF, 1 μg/ml E_2, and 500 ng/ml P_4] for approximately 28 h [7]. Oocytes that matured to metaphase II, as determined by the extrusion of a polar body, were used for ICSI. During the study, ICSI was performed using a piezo drill by a single technician on 147 oocytes, with 103 oocytes from dominant follicles and 44 oocytes from subordinate follicles. In some cases, oocytes from dominant and subordinate follicles of the same mare underwent ICSI at different times. In these cases, two sperm samples were prepared and analyzed individually.

After ICSI, potential zygotes were placed into 30-μl drops of culture medium (Dulbecco Modified Eagle medium/F12 with 10% fetal calf serum) under oil (Ovoil™, Vitrolife, Göteborg, Sweden) at 38 °C in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2. The day after ICSI, potential zygotes were observed at X 200 magnification for cleavage; if cell division had not occurred but signs of imminent cleavage, such as change in shape or fragmentation of the oolemma, were observed, the zygote was reassessed for cleavage on the following day. Cleaved embryos were evaluated on Days 5, 6 and 7 (Day 0 = day of ICSI) for development into a morula or blastocyst. Embryos were considered to be at the morula stage and acceptable for transfer when a compact mass of cells was observed. Embryos were considered to be at the blastocyst stage of development when a defined ring of trophoblast was observed. Embryos (Day 6 or 7) were transferred into the uteri of recipient mares by transcervical

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