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Seminal plasma and seminal plasma proteins added to bulk sorted sperm do not alter the mRNA expression of *in vitro* produced bovine embryos

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Abstract

Although sex-sorted sperm have been used for AI and IVF for over a decade there is still need to improve the technology as the results are highly variable. The goal of the present study was to assess the effect of seminal plasma and seminal plasma proteins as a supplement to sorted sperm on subsequent embryonic development, as a beneficial effect of these substances has been reported. *In vitro* matured oocytes were fertilized *in vitro* with either unsorted sperm (n = 215; Group 1), bulk sorted sperm (n = 226; Group 2), bulk sorted sperm extended in the presence of 1% seminal plasma (n = 185; Group 3) or bulk sorted sperm supplemented with seminal plasma proteins (4 mg mL^{-1}; n = 254; Group 4). An additional group of oocytes (n = 307; Group 5) was fertilized with the semen of another bull routinely used for IVF and served as a laboratory standard control. Subsequently, the presumptive zygotes were cultured for 8 days under standard conditions (SOFaa, 39 °C, 5% CO_2, 5% N_2). Cleavage rates were assessed on day 3 p.i. (post insemination; group 1: 30.5 ± 14.7%; group 2: 28.8 ± 9.8%; group 3: 20.8 ± 14.9%; group 4: 25.7 ± 8.2%; group 5: 54.8 ± 11.5%). Development rates were documented on days 7 p.i. (group 1: 7.3 ± 6.6%; group 2: 5.6 ± 3.1%, group 3: 6.2 ± 7.7%, group 4: 6.7 ± 5.9%, group 5: 20.2 ± 6.9%) and 8 p.i. (group 1: 8.9 ± 7.0%; group 2: 6.0 ± 2.9%; group 3: 8.6 ± 11.3%; group 4: 7.8 ± 6.2%; group 5: 23.3 ± 7.8%), respectively. Significant differences among cleavage and development rates could only be seen for Group 5 compared to all other groups. However, this difference between Groups 1−4 vs. Group 5 regarding the development rates on Day 8 could not be detected when assessing the development rates on base of the number of cleaved embryos instead of the number of oocytes fertilized (group 1: 31.4 ± 17.2%; group 2: 26.0 ± 21.0%; group 3: 33.3 ± 19.05%; group 4: 26.6 ± 17.8%; group 5: 42.6 ± 11.3%). The relative abundance of six different developmentally important gene transcripts (G6PD, HSP1A1, SLC2A3, BAX, BCL2L1, DNMT3A) was determined using single Day 8 expanded blastocysts of all five groups. No significant differences were seen among the embryos of the five groups. Our results show that neither the bulk sorting procedure nor the addition of seminal plasma or seminal plasma proteins, respectively, affected cleavage and development rates when sperm from a specific bull was used. Additionally, sorting and subsequent exposure of sperm to either seminal plasma or seminal plasma proteins did not influence mRNA expression in bovine IVP embryos.

Keywords: IVP; Embryo; Bovine; Sorted semen; mRNA

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1. Introduction

The method of separating X- and Y-bearing sperm has been improved continuously [1] since the first report of live offspring being born following sorting of sperm in 1989 [2]. Nevertheless, there are still difficulties as compared to the use of unsorted semen that may result in reduced fertility in AI and IVF [3].

Alterations in sexed sperm include a reduced lifespan [4], as well as an increased proportion of capacitated sperm [5]. Additionally, sorted sperm seems predisposed to undergo an accelerated acrosome reaction [6]. The nearly complete removal of seminal plasma (SP) during sorting by high dilution results in limited protection of the sperm in the adjoining freezing process [7]. Therefore, seminal plasma and seminal plasma proteins (SPP) have been added to semen of different species at different times during the sorting process and following freezing and thawing process to attenuate the detrimental effects of sorting on sperm. These studies have been published with varying results. It was possible to improve post thaw semen quality if SP was added to boar semen during the thawing process. On the contrary, if SP was added before freezing damaging effects predominated [8]. The supplementation of bull spermatozoa with SP attenuated the dilution effect, described for the cryopreservation process [9]. In ram semen it was possible to reduce post-thaw damage if SPP were added after sorting and before freezing [10]. Seminal plasma proteins [11] were also able to inhibit capacitation-like changes in boar spermatozoa. However, studies focusing on the supplementation of sorted sperm with SP or SPP, respectively, have apparently not been conducted in the bovine.

Sex-sorted sperm have been used in bovine *in vitro* embryo production (IVP) with highly variable results regarding cleavage and blastocyst rates. In some studies, these rates were similar to those obtained succeeding fertilization with unsorted sperm [12,13], whereas others reported lower rates of cleavage and blastocyst formation [14].

Reports regarding the assessment of gene transcripts of bovine embryos produced *in vitro* with sorted sperm are very limited. Changes in spermatozoa because of sorting were transferred to embryos, resulting in alterations in the relative abundance of gene transcripts depending on embryo sex and age [15,16].

The objective of the present study was to determine potential effects of adding seminal plasma or seminal plasma proteins to sorted sperm on quality and developmental ability of bovine IVP embryos at the morphologic and molecular level.

2. Materials and methods

2.1. General remarks

Unless otherwise stated all chemicals were obtained from Sigma (Steinheim, Germany).

2.2. Seminal plasma and seminal plasma proteins

Seminal plasma was collected from bulls with proven high fertility (n = 10). Following the addition of a protease inhibitor cocktail, seminal plasma was separated from semen by centrifugation (6000*g* for 20 min at 4 °C) using a Heraeus centrifuge (Thermo Scientific, Schwerte, Germany). The supernatant were filtered through 0.2 μm cellulose acetate membrane, pooled and stored at −80 °C until used.

Bovine seminal plasma proteins were prepared by ethanol precipitation of bovine seminal plasma. Nine volumes of cold ethanol were added to one volume of seminal plasma and left for 60 min at −20 °C to precipitate the proteins which were then recovered by centrifugation at 10 000*g* for 10 min at 4 °C. The protein pellets were dissolved in Tris-buffer and stored at −80 °C until used. Protein content was determined using a Bio-Rad DC Protein Assay kit with BSA as the standard.

2.3. Semen preparation

Semen was obtained from a bull that in preliminary studies in our laboratory (unpublished) had an acceptable development rates in IVP. This semen was allocated to four treatment groups: Unsorted; bulk sorted; bulk sorted with the addition of seminal plasma (Sorted + SP); and bulk sorted with the addition of seminal plasma proteins (Sorted + SPP). In addition, semen from another bull served as internal control for IVP to guarantee our laboratory standards (Control). These five groups of semen were then used for IVF.

Initially, semen was stained with propidium iodide (P4170, Sigma Aldrich, Taufkirchen, Germany) and Hoechst 33 342. Following an incubation period of 30 min, semen was filtered through a 35-μm cell strainer (Falcon Becton Dickinson, and Co, Franklin Lakes, NY, USA).

A Mo Flo XDP cell sorter (Beckman Coulter, Miami, FL, USA), equipped with an argon 5W UV-Laser (Coherent Laser, Inova 90-C5, Dieburg, Germany) set to a 200-mW output and a Sapphire 488−200CDRH Laser (Coherent Laser, Dieburg Germany) set to 100-mW output was used for all sperm samples subjected to the sorting process. The sorter was operated at 40 psi.