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ACCEPTABLE MICROORGANISMS CONCENTRATION IN A SEMEN SAMPLE FOR *IN VITRO* EMBRYO PRODUCTION

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SHORT COMMUNICATION

ABSTRACT

The aim of this study was to report that the acceptable concentration of microorganisms in a semen sample for insemination may not be safe for an *in vitro* fertilization procedure. It seems that the semen sample should be completely germ-free, because of the excellent microorganism proliferation condition promoted by the in vitro environment.

Key words: semen, microorganisms, *in vitro* fertilization

Fundamental procedures currently used in *in vitro* embryo production (IVP) include transvaginal aspiration and oocytes *in vitro* maturation; spermatozoa *in vitro* capacitation and *in vitro* fertilization (IVF) (4). Infectious agents in IVP systems might reduce the number and the quality of embryos generated and result in transmission of disease to recipients and offspring, or even confound findings for research (3).

Semen is a potential source of non-pathogenic, as well as pathogenic microorganisms. Semen collection can be relatively free of contaminants, but it is not necessarily germ-free (3). Units of semen containing *Stenotrophomonas maltophilia, Pseudomonas putida, Pseudomonas aeroginosa, Enterobacter cloacae, Staphylococcus sciuri, Acinectobacter cacloaceticus, Pantoeau agglomerans and Flavobacterium spp* have been reported as contaminants in IVF embryo cultures (2). The aim of this work was to report that the acceptable concentration of microorganisms in a semen sample for insemination may not be safe for an IVF.

Routine IVF procedures were performed with oocytes collected from ovaries derived from slaughtered cows, and maturated for 24h in 5% CO_2 atmosphere at 39ºC. The semen samples were thawed at 37ºC/30' and processed in Percoll gradient (Cultilab, Campinas, Brazil) for spermatozoa capacitation. After maturation time, the oocytes were washed in Human Tubal Fluid (HTF – Cultilab, Campinas, Brazil), a fertilization medium, supplemented with 10% bovine fetal serum (BFS) and kept with the processed spermatozoa for fertilization (18-21h). However, after fertilization, in two consecutive IVF procedures, performed with two different semen samples that had presented normal evaluation parameters, the presence of contamination was detected. To locate the main focus of contamination, a new IVF procedure was performed, with the same semen samples, following the pattern protocol (1).

One fertilization dish was used for each semen sample test. They were prepared with four HTF medium drops (50 µL) each. In the first drop a conventional IVF procedure was done (*in vitro* matured oocytes with processed spermatozoa), in the drop 2 just oocytes were kept and in the drop 3 just processed semen. In the fourth drop HTF medium was left to be used as negative control. The dishes were maintained in 5% CO_2 environment for 18h. The presence of contamination was confirmed in drops 1 and 3 of each dish, showing that probably the semen was the contamination focus. The drops containing the oocytes and

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the HTF medium were tested in bacterial culture, and presented negative results. However, bacterial culture tests performed in the two semen samples used in the IVF and other seven non-used samples of the same lot indicated the two first semen samples were positive for *Acinetobacter* spp (5000 cfu/ml), *Enterobacter aerogenes (*5000 cfu/ml), *Escherichia coli* (5000 cfu/ml) and *Streptococcus* spp (uncountable cfu/ml). Among the other seven semen samples, three were negative and four were positive for *Acinetobacter* spp (300cfu/ml e 1600 cfu/ml), *Enterobacter aerogenes* (uncountable cfu/ml) and *Alcaligenes faecalis* (uncountable cfu/ml). The presence of those bacteria in the semen samples makes the IVF procedure not viable.

According to these findings, it is important to consider that new animal reproduction techniques brings new challenges for disease control (4). Therefore, it is important to have in mind that the quality control patterns established for old techniques should not be consider safe for the new ones. This study indicates that apparently microorganisms concentration acceptable in semen samples used for artificial insemination should not be the same accepted for IVF procedure. It should be completely germ-free, because *in vitro* system promotes an excellent environment for microorganisms proliferation.

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RESUMO

Concentração de microrganismos aceitável em uma amostra de sêmen para produção de embriões *in vitro*

O objetivo foi relatar que a concentração de microrganismos aceitável para uma amostra de sêmen utilizada para inseminação pode não ser segura para a realização de fertilização *in vitro*. Aparentemente a amostra deve ser isenta da presença de contaminantes, pois a condição *in vitro* promove ambiente favorável para seu crescimento.

Palavras-chave: sêmen, microorganismos, fertilização *in vitro*

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