

EFFECT OF LUBRICANTS DEVELOPED FOR FERTILITY MARKETS ON IN VITRO FERTILIZATION AND EMBRYO DEVELOPMENT

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Abstract

Introduction: Traditional lubricants damage sperm and should not be used while pregnancy is desired. Newer products have been developed for this consumer/patient group. Bovine and human embryos share similar paternal sperm regulatory pathways, making this species a model for detection of sublethal sperm damage (RepBioMedOn 2002:4:170). Objective: Experiments were done to evaluate in vitro fertilization and embryo development following bull sperm exposure to lubricants developed for the fertility market. Methods: Cryopreserved bull sperm was washed, resuspended in medium and placed into one of 5 treatments. These were: 1) Control medium; 2) Pré[®] Lubricant (Pré); 3) FertilityCare[™] (FC); 4) ConceiveEase[™] (CE) or 5) PRE Conceive plus[™] (PC). Lubricants were mixed with sperm at 10% v/v & incubated for 30 min at body temperature. Then sperm from each treatment was placed into fertilization wells with mature bovine oocytes. At 8 hr, putative zygotes were transferred into development medium and further incubated. At 32 hr of culture, dividing embryos were counted (% fertilized oocytes). Results: Embryo development (%) was determined by the number of morulae and blastocysts on Day 7. Friedman's test compared percent fertilization and embryo development in each treatment versus control. Conclusion: Sperm contact with Pré[®] did not interfere with fertilization or embryo development, whereas other lubricants caused significant declines in these end points. The reasons for these differences require additional study.

In Vitro Fertilization & Embryo Development

Treatment	Total Oocytes	% Fertilized Oocytes (±SD)	% Embryos Developing (±SD)	p value fertilization / developed
Control	160	63(8)	45(12)	
Pré	160	62(11)	42(5)	0.900 / 0.470
FC	160	53(12)*	28(20)*	0.003 / <0.0001
CE	160	56(9)*	27(16)*	0.033 / <0.0001
PC	160	56(10)*	28(16)*	0.025 / <0.0001

* denotes treatment means differ from control

Pré[®] Lubricant - INGfertility, Valleyford, WA
 FertilityCare[™] - Marco D'Polo, Ingleburn, NSW, AU
 ConceiveEase[™] - Sepal, Boston, MA
 PREConceive plus[™] - Lake Consumer Products, Inc., Jackson, WI

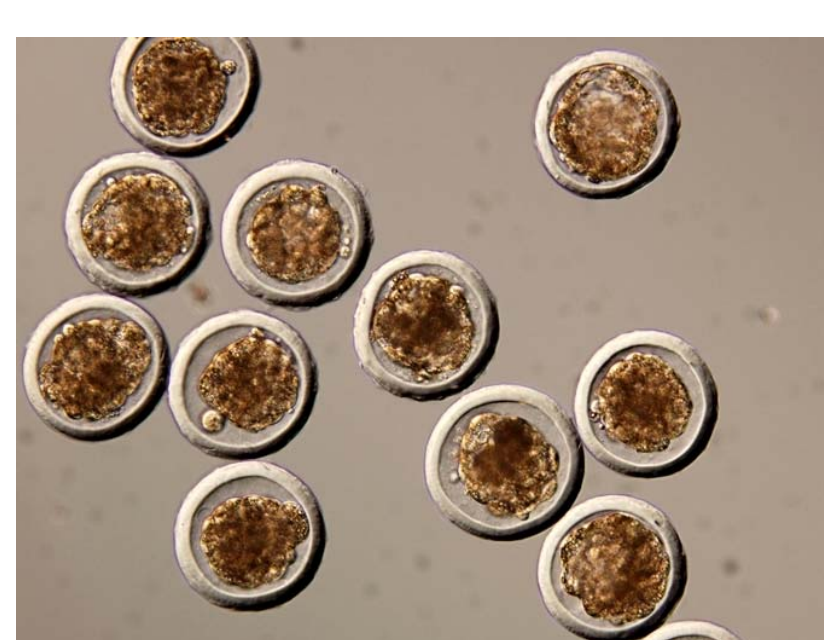
Introduction

Traditional lubricants damage sperm and should not be used when pregnancy is desired. Newer products have been developed for this consumer/patient group. Bovine and human embryos share similar paternal sperm regulatory pathways, making this species a model for detection of sublethal sperm damage (RepBioMedOn 2002:4:170). In addition, federal regulatory agencies have reviewed and accepted the bovine in vitro fertilization and embryo development model for clearance ("approval") of lubricants with human fertility indications such as Pré[®] Lubricant.

Objective

Experiments were done to evaluate animal model in vitro fertilization and subsequent embryo development over 7 days, following pre-fertilization incubation of bull sperm with lubricants developed for the human fertility market.

Normal Day 7 Cattle Embryos



Stage of Normal Development as a Function of Days of Culture for Cattle Embryos

Stage of development	Days of Development
1-cell	0-2
2-cell	1-3
4-cell	2-3
8-cell	3-5
16-cell	4-5
Early morula	5-6
Tight morula	5-7
Early blastocyst	7-8
Blastocyst	7-9

Methods

Methods: Cryopreserved bull sperm was washed, resuspended in TALP medium and placed into one of 5 treatments. These included: 1) Control medium (no lubricant); 2) Pré[®] Lubricant (Pré); 3) FertilityCare[™] (FC); 4) ConceiveEase[™] (CE) or 5) PRE Conceive plus[™] (PC). Lubricants were mixed with sperm in medium at 10% v/v and incubated for 30 min at 39 degrees C. Then sperm from each treatment was transferred into fertilization wells with previously in vitro matured bovine oocytes.

Pré[®] Lubricant - INGfertility, Valleyford WA;
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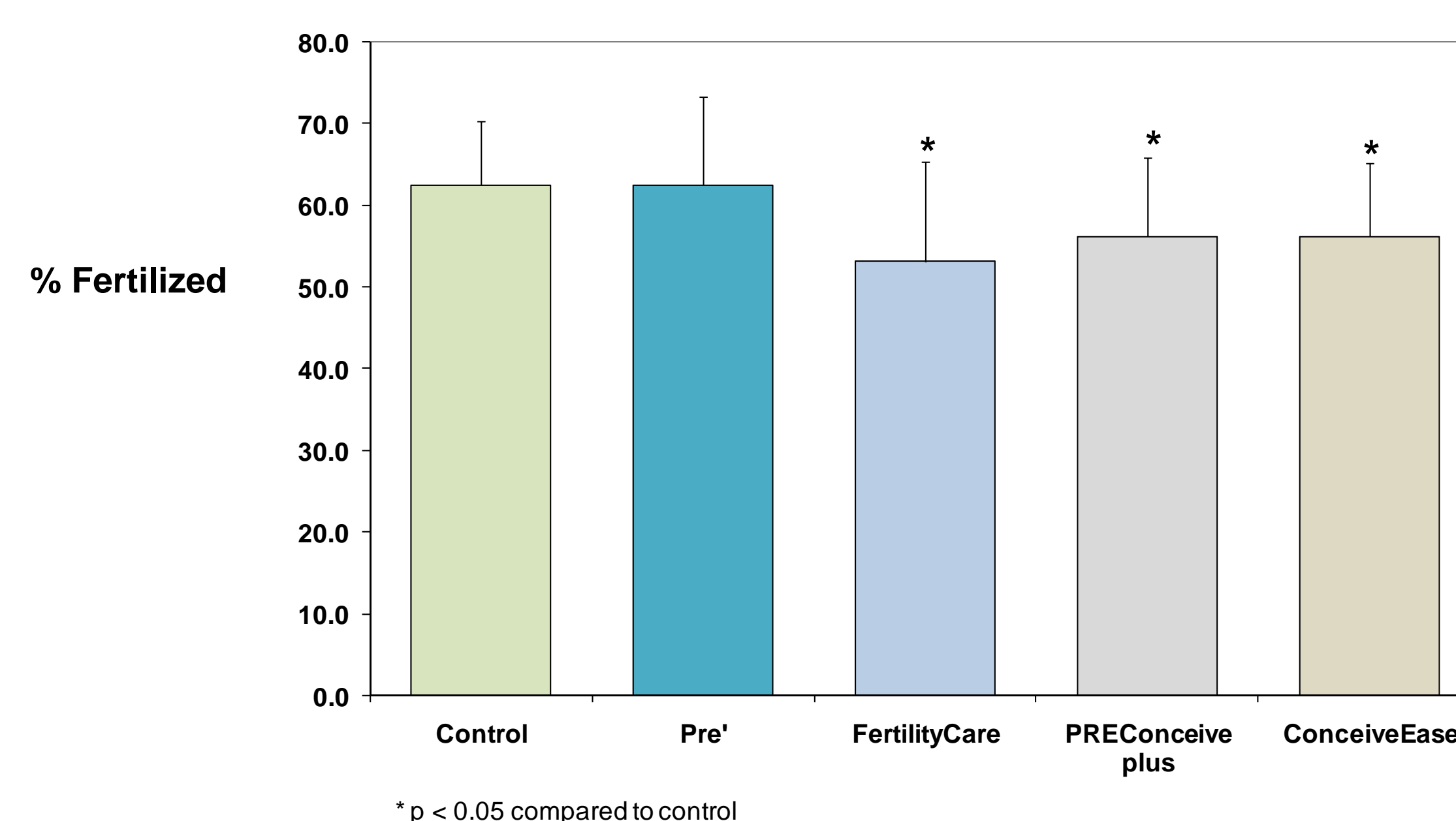
After 8 hrs of fertilization, putative zygotes were transferred into development medium and further incubated. At 32 hr of culture, dividing embryos were counted to determine the percent fertilized oocytes. On Day 7 the number of morulae and blastocysts in each treatment was recorded.

Friedman's test was used to compare fertilization and embryo development rates resulting from sperm exposed to each lubricant versus sperm in the control. Eight replicates were done with twenty oocytes per treatment (n= 160 total oocytes per treatment).

Results

Fertilization rates did not differ between sperm exposed to Pré[®] Lubricant versus sperm in control medium (Figure 1). In contrast, fertilization rates were lower after exposure of sperm to other lubricants (P<0.05) prior to incubation with oocytes. Data expressed as means (±SD).

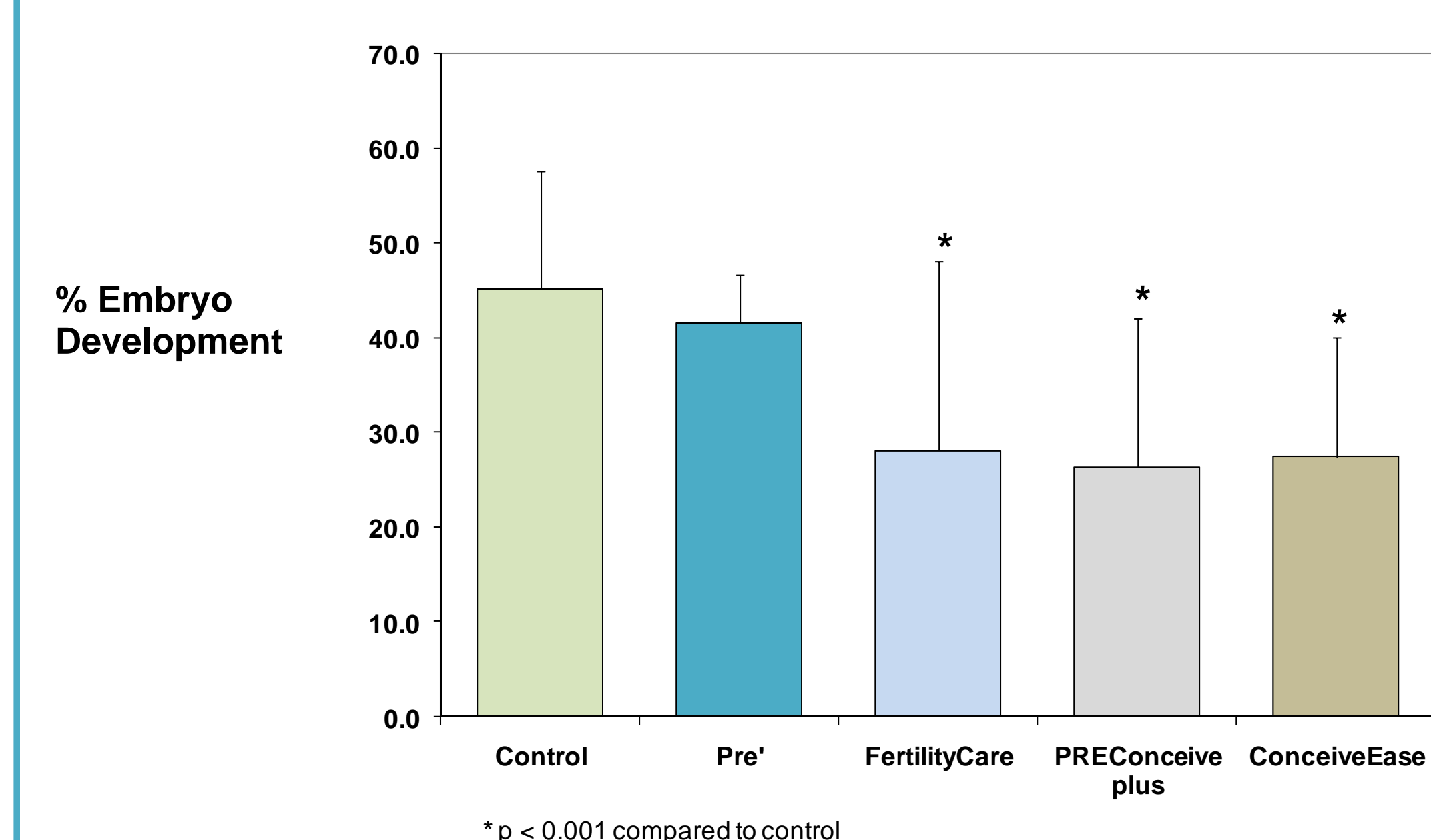
Figure 1



Results (continued)

Embryonic development (Figure 2) was also equivalent following fertilization using sperm incubated with Pré[®] Lubricant or the control (lubricant free medium). In contrast, embryonic development was significantly lower for embryos resulting from sperm incubated with the other lubricant treatments (P<0.005). Data expressed as means (±SD).

Figure 2



Conclusion

Sperm contact with Pré[®] Lubricant did not interfere with bovine in vitro fertilization or embryo development, whereas other lubricants caused declines in these end points. The reasons for these differences require additional study. However, based on timing of embryo failure during development (i.e. prior to morulae stage), damage to the paternal genome resulting from pre-fertilization sperm exposure to some lubricants requires investigation. Certain ingredients may induce damage in sperm, for example the PRE Conceive plus[™] product contains glycerol, ConceiveEase[™] contains mineral oil and FertilityCare's[™] ingredients are unknown. These studies confirm the value of this animal model to detect sublethal sperm damage which may impact subsequent embryo development.

Pré[®] Lubricant is also available as Pré-Seed[®] for intravaginal application.

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