embryos at each time point and blastocysts on Days 6, 7, and 8 from both groups were snap-frozen individually for sexing. Sexing was performed with a single PCR using a specific primer BRY. There was a significantly lower number of cleaved embryos from the 16-h compared with the 24-h maturation group at 28 (10.0 ± 1.51 *v.* 28.8 ± 3.57%), 32 (35.3 ± 1.48 *v.* 57.6 ± 3.33%), 36 (54.8 ± 1.76 *v.* 67.4 ± 2.81%), 40 (63.3 ± 1.82 *v.* 72.0 ± 2.54%), and 48 (70.6 ± 1.78 *v.* 77.1 ± 2.18%) hpi, respectively (mean ± SEM; $P \leq 0.05$). However, the blastocyst yields on Day 6 (17.1 ± 3.11 *v.* 16.4 ± 2.11%), 7 (30.6 ± 4.10 *v.* 34.6 ± 3.51%), or 8 (34.1 ± 3.90 *v.* 39.4 ± 4.26%) were similar for both groups (mean ± SEM; 16 *v.* 24 h, respectively). Significantly more 2-cell early cleaved embryos (up to 32 hpi) were male compared with the expected 1:1 ratio from both groups (16 h: 1.24:0.76 *v.* 24 h: 1.17:0.83, $P \leq 0.05$); however, the overall sex ratio among 2-cell embryos was significantly different from the expected 1:1 in favor of males only for the 16-h group (1.18:0.82, $P \leq 0.05$). The sex ratio of blastocysts on Day 6, 7, or 8 from both groups was not different from the expected 1:1. However, the total number of male blastocysts obtained after 8 days of culture from the 24-h group was significantly different from the expected 1:1 (1.19:0.81, $P \leq 0.05$) and approached significance in the 16-h group. These results show that the maturational stage of the oocyte at the time of fertilization has an effect on the kinetics of early cleavage divisions but not on blastocyst yield. Furthermore, irrespective of the duration of maturation, the sex ratio of early-cleaving 2-cell embryos was weighted in favor of males, and this observation was maintained at the blastocyst stage.

198 FUNCTIONAL EVIDENCE FOR THE EXISTENCE OF AN OVIDUCTAL FACTOR THAT INDUCES ZONA PELLUCIDA HARDENING AND REGULATES POLYSPERMY IN THE PIG AND COW

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Many differences between *in vivo* and *in vitro* fertilization (IVF) efficiency in mammals are related to the differences between IVF media and oviductal fluid. One of the best known examples is the frequency of polyspermy observed under *in vitro* conditions in cattle (Roh S *et al.* 2002 J. Vet. Med. Sci. **64**, 667–671) and, in particular, in pigs (Coy P and Romar R 2002 Reprod. Fertil. Dev. **14**, 275–286). Zona pellucida (ZP) resistance to pronase digestion (ZP hardening) has been considered as a postfertilization event contributing to the block of polyspermy in mammals (Green D 1997 Rev. Reprod. **2**, 147–156). However, pig and cow unfertilized ovulated oocytes show a ZP hardening of hours or days (Katska L *et al.* 1999 Reprod. Dom. Anim. **34**, 255–259; Kolbe T and Holtz W 2005 Theriogenology **63**, 1695–1705) compared with the minutes or seconds observed in the *in vitro*-matured oocytes, even after fertilization (Coy P *et al.* 2002 Reproduction **124**, 279–288; Coy P *et al.* 2005 Reproduction **129**, 19–26). Consequently, we propose the existence of an oviductal factor that induces ZP hardening before any contact of the oocyte with the sperm, thus regulating polyspermy. Porcine and bovine oviductal fluid was obtained by aspiration of oviducts collected at the slaughterhouse and stored frozen. *In vitro*-matured porcine and bovine oocytes were incubated for 30 min in the oviductal fluid, washed thoroughly in fresh medium, and either assessed for ZP digestion time or *in vitro* fertilized. The results, analyzed by ANOVA, showed a very strong ZP hardening in oviductal-treated oocytes (2866.83 ± 94.4 s in the pig and 4301.1 ± 441.7 s in the cow) compared with control oocytes (63.5 ± 2.9 s in the pig and 124.2 ± 5.9 s in the cow). Moreover, the percentage of monospermy for the oviductal-treated oocytes was significantly higher in both species (50.0 ± 10.0% in the pig and 91.7 ± 3.0% in the cow) compared with the control groups (5.56 ± 3.8% in the pig and 80.8 ± 3.5% in the cow). Percentage penetration did not change in porcine oocytes but decreased in bovine oocytes (58.1 ± 3.3 *v.* 38.4 ± 3.3, $P \leq 0.001$), whereas the mean number of sperm per oocyte decreased for the porcine-treated oocytes (2.7 ± 0.2 *v.* 8.2 ± 0.4, $P \leq 0.001$) and did not change for the bovine oocytes. These results support the hypothesis that an oviductal factor induces ZP hardening, contributing to the control of polyspermy in the pig and cow, and that it could be used to improve the output of IVF.

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199 EFFECT OF HEPARIN CONCENTRATION ON BOVINE PREIMPLANTATIONAL DEVELOPMENT *IN VITRO* USING SEX-SORTED SPERM

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The objective of this study was to examine the effect of heparin on bovine IVF and to improve the efficiency of IVF production by using sex-sorted sperm. The fertility performance of sex-sorted and unsorted semen from 4 bulls was compared to determine the optimal heparin concentration during preimplantational embryo development. A total of 7615 matured bovine oocytes were randomly allocated among different heparin concentrations (0, 2.5, 5, 10, 20, 40, 60, 80, and 100 $\mu g\,mL^{-1}$) in Brackett-Oliphant medium and coincubated with either sex-sorted or unsorted sperm for 6 h. Presumptive zygotes were cultured in CR1aa + 6 $mg\,mL^{-1}$ of BSA in 5% O_2 , 5% CO_2 and 90% N_2 at 39°C until Day 8 (Day 0, culture post-IVF). Cleavage rates at Day 2 and embryo development to blastocyst (BL) at Day 8 were recorded. Data (4 replicates) were analyzed by a general linear