injections or diluted in SRF and given by a single injection. In Experiment 1, crossbred beef cows ($n = 11$) and heifers ($n = 5$) were randomly allocated to 3 treatment groups in a crossover design (i.e. all animals were treated 3 times, and all treatments were represented in the 3 replicates). All donors had all follicles >8 mm in diameter ablated by ultrasound-guided follicle aspiration on Day 0, received 500 μg of cloprostenol (prostaglandin $F_{2\alpha}$, Ciclase, Syntex, Argentina), and were treated as follows: Group 1 (multiple FSH): 160 mg of Folltropin-V divided into 4 IM injections administered twice daily (i.e. Days 1 and 2); Group 2 (single FSH): 160 mg of Folltropin-V diluted in 4 mL of 25% SRF and given by a single IM injection in the neck on Day 1; Group 3 (control): no FSH treatment. On Day 4, oocytes were aspirated from all follicles >3 mm in diameter by ultrasound-guided follicular aspiration, classified, matured in TCM-199 supplemented with 0.4% BSA, fertilized in Fert medium, and *in vitro* cultured in SOF supplemented with 0.4% BSA under oil at 37°C, 5% CO_2, 5% O_2, 90% N_2, and saturated humidity for 7 days. Data were analyzed by ANOVA. The mean (±SEM) numbers of follicles aspirated and oocytes recovered were higher ($P < 0.05$) in Groups 1 (multiple FSH, 13.6 ± 1.9 and 8.5 ± 1.0) and 2 (single FSH, 14.7 ± 1.7 and 9.4 ± 1.3) than in Group 3 (control, 8.7 ± 0.9 and 5.8 ± 1.0). The mean number of blastocysts after 7 days in culture was numerically higher ($P = 0.2$) in Groups 1 (2.1 ± 0.7) and 2 (2.6 ± 0.6) than in Group 3 (1.1 ± 0.4). In Experiment 2, lactating Holstein cows ($n = 10$) received 2.5 mg of estradiol benzoate and 50 mg of progesterone IM on Day 0. On Day 4, all cows received prostaglandin $F_{2\alpha}$ and were randomly allocated to 2 treatment groups, to receive 200 mg of Folltropin-V divided into 4 twice-daily IM injections or 200 mg of Folltropin-V diluted in 25% SRF and given as a single IM injection. On Day 7, oocytes were collected and treated like those in Experiment 1. The mean (±SEM) numbers of follicles aspirated (21.2 ± 3.5 and 14.4 ± 2.9), oocytes recovered (10.2 ± 1.2 and 13.8 ± 2.9), and blastocysts produced (4.6 ± 0.7 and 6.0 ± 2.0) did not differ for Groups 1 and 2, respectively ($P = 0.3$). In conclusion, superstimulatory treatment increased the number of cumulus–oocyte complexes available for IVF compared with no treatment, and ovarian superstimulatory treatment may be simplified by the use of a single IM dose of a sustained-release product, compared with multiple doses of a conventional product, without a reduction in the recovery or quality of cumulus–oocyte complexes.

208 *IN VITRO* FERTILIZATION UNDER SIMULATED STRESS AND SUBSEQUENT *IN VITRO* EMBRYO PRODUCTION IN THE PIG

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Fertilization is a crucial step for successful reproduction and can be negatively influenced by stressful situations. It is generally accepted that stress affects reproduction, altering the endocrine profile of the female. An altered hormonal environment where the oocyte is developing could affect critical processes such as fertilization. Using a mixed *in vivo–in vitro* system, we assessed the ability of the oocyte to undergo fertilization and early development after exposure to blood plasma from sows that had experienced simulated stress through repeated injections of adrenocorticotropic hormone (ACTH) before ovulation (known concentrations of cortisol and reproductive hormones as well as exact ovulation time assessed by ultrasonography). Oocytes ($n = 926$, 7 replicates) collected from abattoir ovaries were matured in TCM-199 with BSA supplemented with hormones (10 IE mL^{-1} of pregnant mare serum gonadotropin and 5 IE mL^{-1} of hCG) and insulin-transferrin-selenium (5 μL mL^{-1}) for 24 h, followed by 22 h without supplements. During IVF, gametes were exposed to 10% of pooled plasma ($n = 3$ per treatment) collected approximately 1 h before ovulation from ACTH-treated sows (A group), nontreated control sows (C group), or media with BSA (B group) for 24 h. Fresh semen was added at 5×10^5 cells mL^{-1}. Afterward, the remaining cumulus cells and sperm were removed from oocytes by vortexing (1 min), and presumptive zygotes were placed in culture medium (porcine zygote medium). Cleavage rate was assessed at 48 h post-insemination (hpi) and the embryos ($n = 433$, 7 replicates) were cultured up to Day 7 and stained with Hoechst 33342 (10 μg mL^{-1}) to count the total number of nuclei. In addition, non-cleaved oocytes were stained at 48 hpi with Hoechst to assess sperm-zona binding. Binding to the zona was assessed only in oocytes found to be matured. Statistical analysis was done using Kruskal-Wallis ANOVA and the Mann-Whitney U test. The number of spermatozoa bound to the zona pellucida was higher in the B group, and binding was notably negatively affected in the ACTH group (0.43 ± 0.18, 35.93 ± 2.50, and 3.44 ± 1.04 for the A, B, and C group, respectively; $P < 0.001$). Cleavage rate (over total number of presumptive zygotes) in the A group (30.71 ± 3.76%) was significantly lower than in the control groups (59.93 ± 4.0 and 52.2 ± 5.31% for the B and C group, respectively; $P < 0.01$). Blastocyst rate expressed over the total number of embryos was reduced in the A group (9.40 ± 5.20%) compared with the controls (27.10 ± 5.79 and 25.66 ± 5.28% in the B and C group, respectively; $P < 0.05$). However, no differences were found in the total number of nuclei in the blastocysts. The results suggest that fertilization is a sensitive event that could be negatively influenced by stress, subsequently affecting early embryo development. A reduced number of spermatozoa attached to the zona and a lower number of embryos and lower blastocyst development were observed in the simulated-stress group. Further studies would help to elucidate which (in the oocyte, spermatozoon, or both) mechanisms are being affected by ACTH-simulated stress around fertilization. Data are expressed as mean ± SEM.

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209 EFFECT OF HEAT STRESS ON DEVELOPMENT OF *IN VITRO*-FERTILIZED AND PARTHENOGENETIC BOVINE EMBRYOS

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Heat stress has been a challenge for bovine reproduction in tropical and subtropical environments. Although the role of the oocyte in thermotolerance has been studied, little attention has been paid to the contributions of sperm to embryo resistance to heat shock. The current study aimed to evaluate the development of fertilized and nonfertilized (parthenogenetic) bovine embryos undergoing heat stress during the pre-implantation stage. Cumulus–oocyte complexes obtained from ovaries collected from *Bos indicus* × *Bos taurus* crossbred cows at slaughter were *in vitro* matured with TCM-199 supplemented with 20 μg mL^{-1} of FSH, under 5% CO_2 at 38.5°C for 24 h. Afterward, oocytes were randomly allocated into 2 groups: 1) IVF and

2) PART (chemical activation for parthenogenesis induction). *In vitro*-fertilized oocytes were cultured with 2.0×10^6 Holstein sperm mL^{-1} in Fert-TALP medium supplemented with heparin, for 20 h. For chemical activation, oocytes were activated with calcium ionomycin for 4 min, followed by 6-DMAP for 4 h, both in CR2aa medium supplemented with 0.1% BSA. Presumptive IVF ($n = 1\,262$) or PART ($n = 1\,206$) zygotes were denuded by vortexing and cultured in CR2aa medium with 2.5% of FCS under 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C. At 44 h post-insemination or chemical activation, embryos were exposed to 38.5 or 41°C for 12 h in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. After that, embryos were cultured at 38.5°C under 5% CO_2, 5% O_2, and 90% N_2 until Day 8 post-insemination. Blastocyst rates were evaluated at Day 7 and Day 8 post-insemination and were calculated based on the total number of presumptive zygotes. Blastocysts at 192 h post-insemination or activation were fixed and permeabilized for TUNEL assay (DeadEnd™ Florimetric TUNEL System, Promega, Madison, WI) according to the manufacturer's instructions. The effect of heat stress was compared within groups (IVF or PART) and the data were analysed by ANOVA. As expected, heat stress reduced the blastocyst rate of IVF embryos at Day 7 (24.3 ± 2.0% and 17.4 ± 2.2% for nonstressed and stressed IVF embryos; $P < 0.05$) and at Day 8 (32.4 ± 1.9% and 23.0 ± 2.1% for nonstressed and stressed IVF embryos; $P < 0.01$). However, the effect of heat stress on blastocyst rate of PART embryos was observed only at Day 8 post-insemination (30.0 ± 1.7% and 22.6 ± 2.0% for nonstressed and stressed PART embryos; $P < 0.05$), with no difference in blastocyst rate at Day 7 (21.6 ± 1.5% and 18.2 ± 1.8% for nonstressed and stressed PART embryos; $P > 0.05$). There was no difference in total cell numbers between nonstressed and stressed IVF or PART embryos. Apoptosis cell numbers and the apoptotic cell index were higher ($P < 0.05$) for stressed IVF (18.45 ± 1.24 and 0.16 ± 0.00) and PART (16.40 ± 5.20 and 0.17 ± 0.00) embryos than for nonstressed IVF (13.70 ± 0.75 and 0.13 ± 0.00) and PART (14.15 ± 0.86 and 0.13 ± 0.00) embryos. In conclusion, heat stress can induce apoptosis in both IVF and PART embryos, but its effect on pre-implantation development may occur at earlier stages in IVF embryos when compared with PART embryos.

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210 CO-CULTURE WITH AUTOLOGOUS CUMULUS CELLS SUPPORTS THE INDIVIDUAL DEVELOPMENT OF SINGLY *IN VITRO*-MATURED AND FERTILIZED BOVINE OOCYTES

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The ability to produce embryos singly *in vitro* (*in vitro* production, IVP) would be a useful tool for many purposes. Without the interfering effects of other developing or degenerating oocytes or embryos, such an individual IVP system is the tool of choice for studies on oocyte quality and oocyte–embryo metabolism. Unfortunately, individual IVP in most cases leads to unsatisfactorily low blastocyst rates. Earlier work showed that individual culture of zygotes on a cumulus cell (CC) monolayer resulted in comparable numbers of good-quality embryos, as obtained following regular group culture (Goovaerts *et al.* 2009 Theriogenology **71**, 729–738). However, co-culture with somatic cells is often criticised because of the undefined culture conditions and for sanitary reasons. In the cited study, CC for monolayer production were obtained from a different batch of ovaries. Our specific aim was to use CC from the zygote itself (autologous CC). Grade I COC ($n = 660$) were collected from slaughterhouse ovaries and randomly assigned to 2 treatments (5 replicates): a completely individual 'single-oocyte' IVP protocol, or routine group IVP as a control. Individual maturation (TCM-199 + 20% serum) and fertilization were performed in 20-µL droplets under oil in 24-well plates. Subsequently, each zygote was stripped and cultured in 20 µL of medium (SOF + 5% serum, 90% N_2, 5% CO_2, 5% O_2), to which the autologous stripped CC were added. Group maturation and fertilization were carried out per 100 COC in 500 µL, whereas group culture was performed per 25 zygotes in 50-µL droplets under oil. Cleavage, blastocyst, and hatching rates were determined 2, 8, and 10 days post-fertilization, respectively. Possible effects of the individual and group cultures were evaluated with binary logistic regression (SPSS 15.0, SPSS Inc., Chicago, IL). No interactions between replicate and treatment were found ($P > 0.05$). Although a blastocyst rate of 15.1% was obtained using single IVP, the general efficacy of the single-embryo production system was lower when compared with group culture (Table 1). In conclusion, although developmental competence was impaired using individual IVP, co-culture with autologous cumulus cells can be useful in specific experimental setups in which the influence of other oocytes or embryos or heterologous somatic cells is unacceptable.

Table 1. Cleavage, blastocyst, and hatching rates after individual and group *in vitro* production (IVP)

IVP treatment	No. of oocytes	Cleavage (%)	Blastocysts Day 8/oocytes (%)	Blastocysts Day 8/Cleaved (%)	Hatched (%)
Group	431	313 (67.9)[a]	121 (26.2)[a]	38.7[a]	50.0[a]
Individual	199	109 (54.8)[b]	30 (15.1)[b]	27.5[b]	26.1[b]

[a,b]Different superscripts in the same column indicate a statistical difference ($P < 0.05$).

211 *IN VIVO* MATURATION AND *IN VITRO* FERTILIZATION OF ALPACA OOCYTES

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The objectives of the study were to evaluate the ovarian follicular response, cumulus–oocyte complex (COC) collection rate, fertilization, and culture of COC collected from alpacas after treatment with 2 different gonadotropins. Female alpacas were assigned to Group 1 ($n = 8$), 200 mg of FSH (Folltropin, Bioniche, Belleville, Ontario, Canada) divided b.i.d. for 3 days, plus a single IM dose of 1000 IU of hCG (Chorulon, Intervet, Salamanca,


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