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Use of commercial or handmade well of well (wow) dishes for *in vitro* production of bovine embryos

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The Well of Well (WOW) embryo culture system was developed as an alternative for culturing individual embryos in a single drop. It is used in the handmade cloning technique in which the removal of the zona pellucida (ZP) is required. The individual culture of several embryos in a single drop is also useful for transfection or transduction of zygotes where ZP removal is required. Well of well embryo culture dishes can be acquired on the commercial market or handmade. The aim of this study was to evaluate the efficiency of commercial or handmade WOW dishes in the production of embryos without ZP. Oocytes from ovaries collected at slaughterhouse were selected and in vitro matured in TCM 199 medium (Invitrogen, California, USA) supplemented with 10% of estrus cow serum and 20ug of FSH mL-1 in atmosphere with saturated humidity and 5% of CO_2 in air and 38.5ºC for 24h. The oocytes were in vitro fertilized in FERT-TALP medium with 2x106 spermatozoa mL-1 for 20-22h in the same conditions of in vitro maturation. Following in vitro fertilization, the presumptive zygotes were denuded with hyaluronidase at 0.1% m/v (Sigma, St Louis, USA) by vortexing for 5 minutes, and were randomly divided into three groups. G1 (n=244): cultured in conventional dishes, without ZP removal; G2 (n=132): cultured in commercial WOW dishes, with ZP removal; G3 (n=144): cultured in handmade WOW dishes, with ZP removal. In all groups, we used CR2aa medium supplemented with 2.5% SFB in atmosphere with 5% CO_2, 5% O_2 and 90% N_2. In G2 and G3 groups, ZP was removed with pronase 2mg ml-1 (Sigma). In the G3, the microwells were produced in 35x10mm petri dishes without the culture medium using a glass microneedle with the edge preheated. It was left the smallest space as possible among the microwell, they were covered with CR2 medium and any formed air bubbles inside the microwell were removed. In all groups, 25-30 presumptive zygotes per dish and the medium was covered by mineral oil. For the statistical analysis, data was submitted to the qui-square test ($P<0.05$). There was no difference ($P>0.05$) in cleavage rate between G2 and G3 treatments (91.5 and 88.0%, respectively), but both were higher ($P<0.05$) than G1 (62.5%). The blastocysts rate on day 7 did not differ ($P>0.05$) among groups (24.5, 16.8 and 19.7%, for G1, G2 and G3, respectively). Blastocysts rate on day 8 in G2 (18.4%) was lower ($P<0.05$) than G1 (31.5%), but not different ($P>0.05$) from G3 (24.3%). The percentage of cleaved embryos that developed to blastocyst until day 8 was lower ($P<0.05$) in G2 (19.8%) and G3 (25.6%) compared with G1 (50.5%). We conclude that commercial and handmade WOW dishes allow bovine embryos development without ZP, although WOW system has reduced the proportion of embryos developing toward blastocyst stage compared to conventional embryo culture system.

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