compact cumulus-oocyte complexes were used for the activation treatment. The oocytes were matured for 30 h in TCM 199 with Earle's salts supplemented with 20% FBS, 5 mg/ml FSH, 1 mg/ml oestradiol and cultured at 38.5°C in air containing 5% CO_2. After maturation the oocytes were denuded of cumulus cells by pipetting them in hyaluronidase (300 i.u./ml PBS) and those showing the first polar body were used for the artificial activation treatments and ICSI procedure. Treatment 1–5 μM ionomycin in TCM 199 medium (5 min) + 10 μg/ml cycloheximide in TCM medium + 10%FBS (24 h). Treatment 2–50 μM calcium ionophore in TCM 199 medium (5 min) + 10 μg/ml cycloheximide in TCM medium + 10%FBS (24 h). Treatment 3–5 μM ionomycin in TCM 199 medium (5 min) + 2 mM 6-DMAP in TCM medium + 10%FBS (3.5 h). Treatment 4–50 μM calcium ionophore in TCM 199 medium (5 min) + 2 mM 6-DMAP in TCM medium + 10%FBS (3.5 h). ICSI and 'scheme ICSI' was performed as described previously by Palermo et al. (1992). After artificial activation and ICSI oocytes were transferred into DMEM F 12 medium supplemented with 10% FBS and cultured at 38.5°C in 5% CO_2 in air. The results of this experiment are summarized in Table 1. In the case of chemical activation the embryonic divisions stopped at the 2-cell stage. Therefore, attempts were made to improve efficiency by modifying treatment 3. Concentration of ionomycin was increased to 10 μM and the time of incubation in 6-DMAP was extended to 4 h. Oocyte activation protocols used successfully in other animal species are ineffective in the case of horses. Equine oocytes require stronger stimuli to induce partenogenetic embryos divisions.

Key Words: Equine oocytes, parthenogenesis, artificial activation

Table 1. Efficiency of activation of mare oocytes

Method of activation	No. oocytes	No. 2-blastomers (%)	No. 4-blastomers (%)	Total number of cleaved oocytes (%)
Treatment 1. I.O. + CHX	104	0	0	0
Treatment 2. C.I + CHX	90	0	0	0
Treatment 3. I.O + 6-DMAP	97	17 (17.5)	0	17 (17.5)
Treatment 4. C.I. + 6-DMAP	107	10 (9.2)	0	10 (9.2)
Modified Treatment 3	53	11 (20.7)	8 (15.1)	19 (35.8)
Schame ICSI	76	0	0	0
ICSI	44	9 (20.4)	8 (18.25)	17 (38.65)

I.O., ionomycin; CHX, cycloheximide; 6-DMAP, 6-dimethylaminopurine.

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WTAP gene expression in *in vivo* and *in vitro* porcine oocytes

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The Wilms' tumor 1 suppressor gene WT1 appears to play an important role in both transcriptional and posttranscriptional regulation of cellular genes. WT1 and WT1-associating protein (WTAP) are localized throughout the nucleoplasm as well as in speckles and partially colocalized with splicing factors. WTAP is a factor essential for cyclin A2 mRNA stabilization and regulation of G2/M cell-cycle transition in somatic cells. For cell-cycle regulation of WTAT, WTAP might be involved in regulation of oocyte maturation. The mechanism of WTAP in somatic cells has been previously characterized, however its biological function in oocytes still remains to be elucidated. To investigate the role of WTAP in oocytes, the abundance of its mRNA was examined in *in vivo* and *in vitro* matured porcine oocytes by using deep sequencing. More reads for WTAP mRNA were detected in *in vivo* than in *in vitro* matured oocytes (12.5 vs. 0). The expression of cyclin A2 in *in vivo* was higher than *in vitro* (0.5 vs. 8.4). However, the expression of cell cycle related genes (cyclin B1, cyclin B2, CDC20) in *in vivo* oocyte was lower compared to *in vitro*. WTAP gene expression was confirmed by quantitative real-time PCR. The expression level of WTAP variant 1, WTAP variant 2, and cyclin A2 was compared between oocytes matured *in vivo* and *in vitro*; GAPDH was served as internal control. The mRNA abundance of WTAP variant 2 and cyclin A2 in *in vivo* matured oocytes were significantly higher than *in vitro* ($p < 0.05$). To further characterize porcine WTAP, the entire coding sequence of WTAP1 was amplified by PCR and sequenced; WTAP variant 2 is a shorter version of WTAP variant 1 due to a premature stop codon. The sequencing result indicated that porcine WTAP variant 1 is consist of 395 amino acids and showed high identities against human and mouse WTAP sequence; both 94% each respectively. This is the first report of the presence of WTAP in oocytes. This study demonstrates that WTAP is expressed higher in *in vivo* oocytes compared to *in vitro*. This suggests *in vitro* matured oocytes may show lower developmental competence because of lower level of WTAP thereby unstable expression of cyclin A2 in porcine oocytes. This research would be helpful to improve current *in vitro* maturation system and to characterize oocyte maturation.

Key Words: Pig oocytes, WTAP gene, *in vitro* matuation, cell cycle

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Different media used for selection of competent swine oocytes with Brilliant Cresyl Blue staining

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Selection of more competent oocytes increases *in vitro* embryo production rates. Brilliant Cresyl Blue (BCB) is a noninvasive method for oocyte selection, which estimates cellular viability by measuring the activity of glucose-6-dehydrogenase. Oocytes stained by BCB are considered more suitable for *in vitro* maturation (IVM). PBS is the most common medium used for BCB staining, associated with 90 min incubation. The aim of this study was to test the selection of swine oocytes in a richer medium, with two BCB concentrations and shorter incubation period. The maintenance medium tested was the modified porcine zygote medium (PZM4-m). Prior to IVM, 666 oocytes were distributed in the following groups: (i) porcine fluid follicular (PFF), (ii) PBS, (iii) PZM4-m, (iv) PBS with 13 μM of BCB, (v) PBS with 26 μM of BCB, (vi) PZM4-m with 13 μM, and (vii) PZM4-m with 26 μM of BCB. After incubation for 60 min at 39°C in the different media, oocytes were classified as positive (stained) or negative (no staining), except for group 1 which was washed in PFF and were not incubated, being considered the general control. IVM was performed in NCSU-23 m with eCG, hCG, hypotaurine, β-mercaptoethanol, cysteine, EGF, AMP-c and PFF, in the first 24 h, followed by NCSU-23 without eCG, hCG and AMP-c, for additional 24 h. Nuclear maturation was assessed with Hoechst (Sigma H33342) using epifluorescence microscopy. The results were analyzed by Chi-Square using the Software Statistix 9.0. The rate of metaphase II in group 1 was 72% ($n = 50$); 30% in group 2 ($n = 55$); 55.6% in group 3 ($n = 72$); 67.3% in positive group 4 ($n = 110$); 43.7% in negative group 4 ($n = 48$); 50% in positive group 5 ($n = 44$); 45.5% in negative group 5 ($n = 33$); 48% in positive group 6 ($n = 166$); 35.7% in negative group 6 ($n = 29$); 27.5% in positive group 7 ($n = 51$) and 12.5% in negative group 7 ($n = 8$). These results showed that maturation rates in PBS in both BCB concentrations 13 μM (67.3%) and 26 μM (50%) was statistically superior to PZM4-m, which had 48% in 13 μM and in 27.5% in 26 μM of BCB. Maturation rates in PBS with 13 μM (67.3) of BCB were superior ($p < 0.05$) than in 26 μM (50%). However, maturation rates in PBS with 13 μM of BCB (67.3%) were lower ($p < 0.05$) than in the general control group (72%). This research showed that is possible to decrease the BCB incubation period from 90 to 60 min in order to obtain effective oocyte coloration. Regarding the tested media PBS was superior to PZM4-m and a lower BCB concentration 13 μM was better than 26 μM. Therefore, both PBS and PZM4-m with BCB showed lower MII rates than control group. Further studies are needed to more precisely define the effectiveness of PZM-4m as adilution media for BCB staining.

Key Words: Oocyte, *in vitro* maturation, Brilliant Cresyl Blue, PZM4-m

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