

- Pandey A.N., Tripathi A., Premkumar K.V., Shrivastav T.G., Chaube S.K. 2010. Reactive oxygen and nitrogen species during meiotic resumption from diplotene arrest in mammalian oocytes. *J. Cell. Biochem.* 111 : 521–528.
- Petr J., Krejčová M., Rajmon R., Jílek F. 2011. Activation of protein kinase C suppresses fragmentation of pig oocytes aged *in vitro*. *Animal* 5 : 565–571.
- Pires P.R., Santos N.P., Adona P.R., Natori M.M., Schwarz K.R., de Bem T.H., Leal C.L. 2009. Endothelial and inducible nitric oxide synthases in oocytes of cattle. *Anim. Reprod. Sci.* 116 : 233–243.
- Premkumar K.V., Chaube S.K. 2015. Nitric oxide signals post-ovulatory aging-induced abortive spontaneous egg activation in rats. *Redox Rep.* 20 : 184–192.
- Secondo A., Sirabella R., Formisano L., D'Alessio A., Castaldo P., Amoroso S., Ingleton P., Di Renzo G., Annunziato L. 2003. Involvement of PI3'-K, mitogen-activated protein kinase and protein kinase B in the up-regulation of the expression of nNOSalpha and nNOSbeta splicing variants induced by PRL-receptor activation in GH3 cells. *J. Neurochem.* 84 : 1367–1377.
- Takahashi T., Igarashi H., Kawagoe J., Amita M., Hara S., Kurachi H. 2009. Poor embryo development in mouse oocytes aged *in vitro* is associated with impaired calcium homeostasis. *Biol. Reprod.* 80 : 493–502.
- Tanghe S., Van Soom A., Nauwynck H., Coryn M., de Kruif A. 2002. Minireview: functions of the cumulus oophorus during oocyte maturation, ovulation, and fertilization. *Mol. Reprod. Develop.* 61 : 414–424.
- Tatone C., Amicarelli F., Carbone M.C., Monteleone P., Caserta D., Marci R., Artini P.G., Piomboni P., Focarelli R. 2008. Cellular and molecular aspects of ovarian follicle ageing. *Hum. Reprod. Update.* 14 : 131–142.
- Tatone C., Carbone M.C., Gallo R., Delle Monache S., Di Cola M., Alesse E., Amicarelli F. 2006. Age-associated changes in mouse oocytes during postovulatory *in vitro* culture: possible role for meiotic kinases and survival factor BCL2. *Biol. Reprod.* 74 : 395–402.
- Te Velde E.R., Pearson P.L. 2002. The variability of female reproductive ageing. *Hum. Reprod. Update* 8 : 141–154.
- Tian X.C., Lonergan P., Jeong B.S., Evans A.C., Yang X. 2002. Association of MPF, MAPK, and nuclear progression dynamics during activation of young and aged bovine oocytes. *Mol. Reprod. Develop.* 62 : 132–138.
- Vega C., Moreno-Carranza B., Zamorano M., Quintanar-Stéphano A., Méndez I., Thebault S., Martínez de la Escalera G., Clapp C. 2010. Prolactin promotes oxytocin and vasopressin release by activating neuronal nitric oxide synthase in the supraoptic and paraventricular nuclei. *Amer. J. Physiol. Regul. Integr. Comp. Physiol.* 299 : R1701–R1708.
- Wu Y., Wang X.L., Liu J.H., Bao Z.J., Tang D.W., Wu Y., Zeng S.M. 2011. BIM EL-mediated apoptosis in cumulus cells contributes to degenerative changes in aged porcine oocytes via a paracrine action. *Theriogenology.* 76 : 1487–1495.
- Zhang G.M., Gu C.H., Zhang Y.L., Sun H.Y., Qian W.P., Zhou Z.R., Wan Y.J., Jia R.X., Wang L.Z., Wang F. 2013. Age-associated changes in gene expression of goat oocytes. *Theriogenology.* 80 : 328–336.
- Zhu J., Zhang J., Li H., Wang T.Y., Zhang C.X., Luo M.J., Tan J.H. 2015. Cumulus cells accelerate oocyte aging by releasing soluble Fas ligand in mice. *Sci. Rep.* 5 : 8683.

THE ROLE OF DIFFERENT NO-SYNTASE ISOFORMS IN REGULATORY EFFECTS OF PROLACTIN AND GROWTH HORMONE ON THE STATE OF CHROMOSOMES IN MATURED OOCYTES AGING *IN VITRO*

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Fertility of mammalian females depends on the oocyte quality that declines during biological aging. We have previously found the inhibiting action of two related pituitary hormones, prolactin (PRL) and growth hormone (GH), on destructive modifications of metaphase chromosomes in bovine (*Bos taurus taurus*) ova aging *in vitro*. In the present work, a participation of different NO-synthase (NOS) isoforms in implementation of PRL and GH effects on age-related changes of MII chromosomes during the prolonged culture of *in vitro* matured bovine oocytes was studied. In the absence of the hormones, NPLA, an inhibitor of neuronal NOS (nNOS), did not affect the frequency of chromosome abnormalities in aging oocytes. At the same time 1400W, an inhibitor of inducible NOS (iNOS), and L-NAME, an effective inhibitor of endothelial NOS (eNOS) and nNOS, decreased this frequency, whereas blocking of all three isoforms of NO-synthase led to the opposite effect. The inhibitory effect of PRL on destructive modifications of chromosomes in aging ova increased in the presence of L-NAME but was not expressed when inhibiting nNOS, iNOS or simultaneously all NO-synthase isoforms. Neither NPLA or L-NAME modulated the suppressing action of GH on abnormal changes of oocyte chromosomes. In contrast, blocking of iNOS, as well as all three isoforms of NO-synthase resulted in an increase in the proportion of oocytes with destructive changes in M-II chromosomes. Meanwhile, the level of the total activity of NO-synthase in oocytes did not depend on the presence of the investigated hormones or NOS inhibitors during the prolonged culture of cumulus-oocyte complexes. The findings permit to assume that the inhibiting effect of PRL and GH on abnormal changes of metaphase chromosomes in aging bovine ova is related to a reduction in the activity of endothelial NO-synthase and also, in the case of PRL, to an increase in the activity of neuronal NO-synthase in cumulus cells surrounding oocytes.

Keywords: oocyte aging, metaphase chromosomes, prolactin, growth hormone, NO-synthase, cumulus cells