Effect of combined treatment with GnRH and a non-selective inhibitor of phosphodiesterase (IBMx) on the fertility of rabbit does

Xylouri-Fragkiadaki E., Fotopoulou E., Birkos S., Kalogiannis D., Kotsampasi B., Balaskas C., Chadio S.

Department of Anatomy and Physiology of Domestic Animals, Agricultural University of Athens, Greece

Corresponding Author: Stella Chadio, Department of Anatomy and Physiology of Domestic Animals, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, 75 Iera odos, 11855 Athens, Greece - Tel: +30 2105294428 - Fax: +30 2105294388 - Email: shad@aua.gr

ABSTRACT – The aim of the present study was to investigate whether the addition of a non-selective inhibitor of phosphodiesterase (3 isobuty-1-methylxanthine, IBMx) to GnRH during artificial insemination (AI) could improve reproductive performances of rabbit does. Two series of experiments were carried out in nulliparus (n=120) and multiparus (n=120) rabbit does which were divided in three groups (n=40). The first group (C) received i.m GnRH plus 1 ml saline, the second group (A) received i.m. GnRH plus 50μg IBMx and the third group (B) i.m. GnRH plus 500μg IBMx. Two hours after AI serum was collected for progesterone determination. Seventy two hours after AI six multiparus animals per group were euthanized and their ovaries were preserved for histological analyses. Both pregnancy and birth rates were significantly higher ($p<0.05$) in nulliparus does that received treatment with IBMx (A: 94.7% and 89%; B: 92.5% and 87.5%) than controls (75% and 70%, respectively). Significant difference for litter size of young does was detected between control (8.57±0.477) and treated groups (A:9.97±0.425 and B:10.34±0.425, $p<0.05$). A significant difference ($p<0.05$) was also detected for progesterone levels between control and group B (5.96±1.15 *vs*. 9.15±1.15 ng/ml, respectively). In multiparus does, the number of primary follicles was significantly greater in group B than C (17.25±2.8 *vs*. 7.2±2.8, $p<0.05$, respectively). In view of their marked synergism with hormones that signal through cAMP, phosphodiesterases inhibitors may be used to improve ovulation rate as part of an assisted reproduction technology protocol.

Key words: GnRH, Phosphodiesterase inhibitor, Progesterone, Does fertility.

INTRODUCTION – It is well documented that cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are involved in the control of reproductive processes, by influencing ovarian cell proliferation, apoptosis, secretory activity and oocyte maturation (Conti, 2002; Sirotkin *et al*., 2000). Moroever, cAMP is a potent promoter of GnRH (Poon *et al*., 2008), while both cAMP and cGMP are known mediators of action of LH-RH (Sirotkin *et al*., 1994; Ramakrishnappa *et al*., 2005) and gonadotropins (Sirotkin *et al*., 1994, Hillier, 2001; McKenna *et al*., 2005) and their increased accumulation could promote the action of these hormones in the ovary. Both cyclic nucleotides are hydrolysed by the enzymes - phosphodiesterases (PDEs). It has been shown that synthetic PDE4 inhibitors increased cAMP accumulation in ovarian cells, as well as number of ovulations, embryos and

born pups in gonadotropin-stimulated rats (McKenna *et al*., 2005). These findings indicate that PDEs and other activators of cyclic nucleotide-dependent signaling pathways could be potent promoters of ovarian functions or even alternative to classical hormonal stimulators of reproductive processes. The aim of the present study was to examine the effects of IBMx inhibitor of cAMP and cGMP PDEs on the reproductive efficiency and fertility in rabbits, whose ovarian cycle and ovulation was induced by GnRH.

MATERIALS AND METHODS – Two series of experiment were conducted, the first with nulliparus animals of 4.5 months of age and the second with multiparus animals two years old. In the first experiment 120 rabbits of New Zealand White origin were divided in three groups, (n=40) and subjected to artificial insemination (A.I) by using fresh diluted semen. Immediately after insemination, rabbits were injected i.m with 0.2 ml GnRH (Buserelin, Receptal, Intervet, 4μg/ml/animal). The first group (Control) received GnRH plus 1 ml saline. The second group (A) received GnRH plus 50μg IBMx (Sigma, St.Louis, USA) and the third group was injected with GnRH plus 500μg IBMx. Two hours after treatment sera were collected for progesterone determination. In the second experiment animals were treated exactly as in the first experiment and seventy two hours after A.I., six animals per group were euthanized and their ovaries were collected and preserved for histological analysis. The percentage of pregnant animals was determined by abdominal palpation 12 days after insemination. Birth rate and litter size were determined after partum.
All experiments were carried out with the approval of ethical commission in accordance to Greek and EU regulations concerning animal experiments.

RESULTS AND CONCLUSIONS – Pregnancy rate was significantly higher in nulliparous does that received treatment with IBMx (A:94.7%, B:92.5%) compared to Control (C:75%, $p<0.05$). The same was the case with birth rate which was greater in treated animals (A:89%, B:87.5%) compared to Control (C:70%, $p<0.05$). Significant difference for the litter size of nulliparous does was detected between Control (8.57±0.477) and groups received IBMx (A:9.97±0.425 and B:10.34±0.425, $p<0.05$). A significant difference was also detected for progesterone levels between Control (5.96±1.15) and group injected with 500 μg IBMx (9.15±1.15 ng/ml, $p<0.05$). Pregnancy rate, birth rate as well as litter size in multiparus animals were numerically higher in treated groups, although not reaching significance, indicating that older animlas respond differently to the treatment, probably due to decreased cAMP levels in aging ovary, as it has been reported for rats (Wang *et al*., 2008). Also, progesterone concentrations showed no difference between Control and groups injected with either 50μg or 500μg IBMx (A:10.29±1.669 ng/ml, B:9.085±1.669 ng/ml) compared to Control (10.385±1.669 ng/ml). Histological examination of the ovaries revealed that administration of IBMx affected both number and proportion of follicles of different classes. At the dose of 500μg/animal, an increase in the total number of follicles was detected in the ovary, which was due to an increase in number and proportion of primary follicles (17.25±2.8 *vs* 7.2±2.8, $p<0.05$). The results of the present study are in agreement with previous reports on the involvement of cAMP and their downstream signalling pathways in control of ovarian cell functions (Makarevich and Sirotkin, 2000; Hunzicker-Dunn and Maizels, 2006) and further confirm previously obtained results *in vitro* with pefused rabbit ovary, which showed that co-administration of LH plus IBMx

induced higher cAMP release and resulted in a higher ovulation rate (Holmes *et al*., 1989). Furthermore, present observations, are in good agreement with the results obtained in FSH-primed rats, in which co-administration of HCG with a selective PDE4 inhibitor resulted in enhanced ovulation response (McKenna *et al*., 2005). Treatment with IBMx resulted in an increase in the number of primary follicles. It has been proposed that high levels of intracellular cAMP may enhance steroidogenesis and at the same time induce apoptosis in granulosa cells which may be related to recruitment of primordial follicles, initiation of folliculogenesis and preparation for the subsequent ovarian cycle (Wang, 2006). Therefore, IBMx can promote both recruitment of primary follicles into the reproductive cycle and their atresia, mainly associated with follicle luteinisation. In view of their marked synergism with hormones that signal through cAMP, PDE inhibitors may be used to augment hormonal stimulation and may be suitable for the induction of ovulation in animal production, or/and as part of an assisted reproduction technology protocol in both human and animals.

REFERENCES – **Conti**, M., 2002. Specificity of the cyclic adenosine 3',5'-monophosphate signal in granulosa cell function. Biol. Reprod., 67:1653-1661. **Hillier**, S.G., 2001. Gonadotropic control of ovarian follicular growth and development. Mol. Cell. Endocrinol. 179:39-46. **Holmes**, P.V., Hedin, L., Janson, P.O., 1986. The role of cyclic adenosine 3'.5'- monophosphate in the ovulatory process of the in vitro perfused rabbit ovary. Endocrinology 118:2195-2202. **Hunzicker-Dunn**, M., Maizes, E.T., 2006. FSH signaling pathways in immature granulosa cells that regulate target gene expression: branching out from protein kinase A. Cell Signal. 18:1351-1359. **Makarevich**, A.V., Sirotkin, A.V., 2000. Presumptive mediators of growth hormone action on insulin-like growth factor I release by porcine ovarian granulosa cells. Biol. Signals Recept. 9:248-254. **McKenna**, S.D., Pietropaolo, M.E., Tos, A., Clark, D., Fischer, Kagan, D., 2005. Pharmacological inhibition of phosphodiesterase 4 triggers ovulation in follicle-stimulating hormone-primed rats. Endocrinology 146:208-214. **Poon**, S.L., An, B.S., So, W.K, Hammond, G.L., Leung, P.C., 2008. Temporal recruitment of transcription factors at the 3',5'-cyclic adenosine 5'-monophosphate-response element of the human GnRH-II promoter. Endocrinology 149:5162-5171. **Ramakrishnappa**, N., Rajamahendran R., Lin Y.M., Leung P.C., 2005. GnRH in non-hypothalamic reproductive tissues. Anim Reprod Sci. 88:95-113. **Sirotkin**, A.V., Makarevich, A.V., Genieser, H.G., Kotwica, J., Hetenyl, L., 2000. Effect of four cGMP analogues with different mechanisms of action on hormone release by porcine ovarian granulosa cells in vitro. Exp. Clin. Endocrinol. Diabetes 108:214-219. **Sirotkin**, A.V., Nitray, J., Kolena, J., Bulla, J., 1994. Cooperation between LH-RH and LH in the direct action on the ovary: LH-RH stimulation of LH/hCG receptors, basal and LH-induced cAMP and cGMP release by porcine granulosa cells in vitro. Cell Signal. 6:135-140. **Wang**, Z., Shi F., Jiang, Y., Lu, L., Wang, H., Watanabe, G., Taya, K., 2007. Changes of cyclic AMP levels and phosphodiesterase activities in the rat ovary. J. Reprod. Dev. 53:717-725.