Slovak J. Anim. Sci., 41, 2008 (2): 57 - 59

EFFECT OF 3-ISOBUTYL-1-METHYL-XANTHINE ON RABBIT SUPEROVULATION AND EGG RECOVERY

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ABSTRACT

The aim of our study was to examine the influence of 3-isobutyl-1-methyl-xanthine (IBMX), inhibitor of cAMP and cGMP phosphodiesterases on the reproductive efficiency of gonadotropin-stimulated rabbits. Ovarian cycle and ovulation of control rabbits were induced by PMSG followed by hCG administration. Experimental animals received PMSG and hCG together with IBMX (at dose 5 or 25 mg/animal). After ovulation and mating, the animals were killed; the pronuclear stage eggs were flushed from the oviducts and cultured up to blastocyst stage. Numbers of ovarian *corpora lutea*, ovulated oocytes and oocyte-derived embryos reaching blastocyst stage were determined. It was observed, that injections of IBMX at dose of 5 mg/animal significantly increased all these parameters. IBMX, when injected at dose of 25 mg/ml, increased only the number of developed embryos, but not the number of ovulations and ovulated oocytes. These data demonstrate that IBMX can enhance the stimulatory effect of gonadotropins on the rabbit ovulation, oocyte maturation, and embryo yield.

Key words: gonadotropin, 3-isobutyl-1-methyl-xanthine (IBMX), ovulation, embryo development, rabbit

INTRODUCTION

The involvement of cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), in control of reproductive processes is well documented. Both cAMP (Makarevich and Sirotkin, 2000; Conti, 2002; Mehlmann, 2005; Sirotkin, 2005; Hunzicker-Dunn and Maizels, 2006) and cGMP (Sirotkin et al., 2000; La Polt et al., 2003) play an important role in control of ovarian cell proliferation, apoptosis, secretory activity, oocyte maturation and in mediating the effect of hormonal stimulators on these processes. Both cyclic nucleotides are hydrolysed by the enzymes - phosphodiesterases (PDEs). Mice, deficient in cAMP-specific phosphodiesterases - PDE3A and PDE4, have impaired differentiation of ovarian cells, their response to gonadotropin, oocyte maturation, ovulation and fertility (Conti, 2002; Park et al., 2003; Masciarelli et al., 2004). On the other hand, 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 suggest that PDEs could be potent regulators of reproductive processes in laboratory rodents. Moreover, it is not to be excluded, that synthetic PDEs could be stimulators of reproduction in human and veterinary medicine and animal production. Nevertheless, effects of cAMP- and cGMP-specific PDEs on reproduction of other mammalian species, especially in farm animals, have not been reported yet.

The aim of our study was to examine the influence of 3-isobutyl-1-methyl-xanthine (IBMX), inhibitor of cAMP and cGMP phosphodiesterases on the fertility in rabbits, whose ovarian cycle and ovulation was induced by gonadotropins. The numbers of ovarian *corpora lutea*, ovulated oocytes and oocyte-derived embryos reaching blastocyst stage were determined.

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Received: January 8, 2008; Accepted: January 28, 2008

MATERIALS AND METHODS

Animals and their zygotes were treated as described previously (Chrenek et al., 1999). Briefly, female non-cycling New Zealand White rabbits, 3 months of age, were bred and kept in individual cages under standard conditions at the local rabbit farm of Research Institute of Animal Production, Nitra, Slovakia. Three days before mating, rabbits were treated with pregnant mare serum gonadotropin (PMSG, Werfaser, Alvetra und Werfft AG., Vienna, Austria, 100 IU/animal) followed after 72 h by human chorionic gonadotropin (hCG, Werfachor, Alvetra und Werfft AG, 200 IU/animal). Control animals were treated only with these gonadotropins, whilst experimental females received gonadotropins together with IBMX (ICN, Irvine, CA, USA, at 5 or 25 mg/animal). Gonadotropins were dissolved in PBS immediately before the injection. Stock solution of IBMX (at 1g/ml) was prepared in DMSO and was dissolved in PBS immediately before the experiment, so that the final concentration of DMSO did not exceed 0.001 %. Control animals received 0.001% DMSO in PBS without IBMX. All substances (0.7 ml solution of gonadotropin with IBMX per animal) were injected intramuscularly. At 19-20h after mating, females were killed by decapitation, numbers of *corpora lutea* in both ovaries were determined by visual inspection, the pronuclear stage zygotes were flushed from the oviduct with PBS supplemented with 10% FCS (Gibco BRL/Invitrogen Corp.,Carlsbad, CA, USA) and washed in CIM medium +10% FCS (Gibco BRL, USA). The eggs were cultured in k-DMEM + 10% FBS (Gibco BRL/Invitrogen Corp., USA) at 5% CO_2 and 39°C up to blastocyst cell stage. Stages of embryogenesis were determined under a stereomicroscope at 40- or 100-fold magnification. All experiments were carried out with the approval of a local ethical commission in accordance to Slovak and EU regulations concerning animal experiments.

Each experiment was performed on 1-2 control animals and 1-3 animals subjected to IBMX injections. Each experiment was replicated 4-5 times. Significant differences between the groups were evaluated using one-way ANOVA followed by paired t-test using Sigma Plot 9.0 statistical software (Systat Software, GmbH, Erkrath, Germany). Differences from controls at $P<0.05$ were considered as significant.

RESULTS

A treatment of females with gonadotropins and subsequent mating induced development and ovulation of ovarian follicles, formation of *corpora lutea*, expulsion of oocytes into the oviduct, and subsequent embryo development *in vitro*. The IBMX treatment was able to influence all these processes. IBMX injections at dose of 5 mg/animal significantly increased the number of ovulations/*corpora lutea*, harvested zygotes and embryos derived from these zygotes (Table 1).

Injections of IBMX at higher dose (25 mg/animal) increased number of corpora lutea and harvested eggs too, but the differences between the groups were statistically insignificant. Nevertheless, IBMX at dose of 25 mg/animal significantly increased the number of developed embryos (Table 2).

Table 1: The effect of IBMX injections at dose of 5 mg/animal on some reproductive parameters of rabbits treated with PMSG and hCG

Treatment	No. experiments	No. treated animals	No. *corpora lutea* per animal	No. collected zygotes per animal	No. developed embryos per animal
Control	5	7	15.7±2.4	15.7±2.4	14.4±2.2
IBMX 5 mg/animal	5	11	24.2±1.7	24.2±1.7	23.6±1.5
Differences between the groups			p<0.05	p<0.05	p<0.01

Values are means ± S.E.M.

Table 2: The effect of IBMX injections at dose of 25 mg/animal on some reproductive parameters of rabbits treated with PMSG and hCG

Treatment	No. experiments	No. treated animals	No. *corpora lutea* per animal	No. collected zygotes per animal	No. developed embryos per animal
Control	4	6	22.0±2.9	21.7±3.0	19.7±2.5
IBMX 25 mg/animal	4	12	27.7±3.4	27.7±3.4	27.2±3.4
Differences between the groups			Not significant	Not significant	p<0.05

Values are means ± S.E.M.

DISCUSSION

Our observations confirm the data obtained on rats (McKenna et al., 2005), that PDE inhibitors could be stimulators of fertility, which could, in addition to- and even instead of gonadotropins, promote rodent ovarian folliculogenesis, ovulation, oocyte maturation, fertility and embryo production. Present observations are the first demonstration of the involvement of cyclic nucleotides-dependent intracellular mechanisms in control of rabbit reproductive processes *in vivo*. These new findings are in line with previous reports on the involvement of cAMP (Makarevich and Sirotkin, 2000; Conti, 2002; Mehlmann, 2005; Sirotkin, 2005; Hunzicker-Dunn and Maizels, 2006), cGMP (Sirotkin et al., 2000; La Polt et al., 2003) and their downstream signalling pathways in control of ovarian cell functions. Furthermore, present observations, together with the data obtained previously on rat (McKenna et al., 2005) suggest, that PDE blockers could be used as potent alternative stimulators of the fertility in animal and human assisted reproduction, as well as for the treatment of reproductive disorders in human and veterinary medicine. This is the first demonstration of beneficial influence of the PDE blocker on farm animal reproduction. Nevertheless, prior to practical application of synthetic PDE blockers in control of animal and human reproduction and fertility, some key questions should be addressed. PDE blockers should be tested on other species (especially on large farm animals and human). Possible side-effects should be carefully examined in large-scale and long-term studies. Furthermore, IBMX represents non-specific inhibitor of cAMP and cGMP PDEs. More specific PDE blocker targeting particular cyclic nucleotide could be more efficient. Therefore, further studies including comparison of effects of PDE blockers with different structure, targets and mechanisms of action on different tissues and processes within the reproductive system in different species are required. We may conclude that:

1.IBMX is a potent stimulator of the rabbit fertility, which can increase the number of ovulations/ *corpora lutea*, harvested zygotes and embryos.

2.IBMX, when injected at dose of 5 mg/animal is more efficient, than IBMX injected at dose of 25 mg/ml.

3.A practical application of the IBMX preparation in assisted reproduction, animal production, human and veterinary medicine requires further studies.

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