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Gonadotropin Hyperstimulation Influences the ³⁵S-Methionine Metabolism of Mouse Preimplantation Embryos

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The effects of gonadotropin stimulation on mouse embryo uptake and incorporation of ³⁵S-methionine were studied. We found that the uptake of ³⁵S-methionine was reduced in embryos of stimulated females in both the two-cell and the blastocyst developmental stage. The incorporation of ³⁵S-methionine into protein was not statistically significantly different between the embryos of stimulated and those of unstimulated females. Qualitatively, protein synthesis was equal in both groups as determined with one-dimensional SDS-PAGE. The results are discussed and we conclude that mouse embryo viability in vivo is decreased by ovarian stimulation.

KEY WORDS: mouse embryos; in vivo; gonadotropin hyperstimulation; ³⁵S-methionine metabolism.

INTRODUCTION

Gonadotropin hyperstimulation (stimulation) is the usual tool to obtain higher numbers of follicles and oocytes than in the natural estrous cycle. There are several stimulation strategies. Laboratory rodents are normally stimulated with a combination of human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG) or analogous gonadotropins.

During our investigations concerning the effects of different culture conditions on mouse embryo development, we found a remarkable difference in ³⁵S-

methionine metabolism between in vivo developed embryos obtained from stimulated and those from unstimulated mouse females. The results are presented and discussed in this short report.

MATERIALS AND METHODS

Female Swiss mice were stimulated with hMG (Humegon; Organon, Oss, The Netherlands) and hCG (Pregnyl; Organon) as described before (1). Both stimulated and unstimulated females were placed overnight with a male. The next morning, mated females were separated, and after 24 hr (two-cell embryos) or 96 hr (blastocysts) these females were killed and the oviducts were flushed. The embryos were incubated (1.5 hr, 5% CO₂, 37°C) in human tubal fluid medium supplemented with 0.5% bovine serum albumin (HTF-BSA) and 1.25 mCi/ml ³⁵S-methionine (Amersham, Den Bosch, The Netherlands). After this incubation, the embryos were transferred to electrophoresis lysis buffer (total volume, 20 μl). The uptake and the incorporation in protein of the ³⁵S-methionine were determined by liquid scintillation counting of 2 μl of the lysate and the trichloroacetic acid-precipitable fraction of 2 μl of the lysate respectively. The polypeptides in the lysate of 10 embryos of each group were separated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described before (2) on 4 to 20% gradient prefab gels (Biorad, Veenendaal, The Netherlands).

Three replicate experiments were performed with two or three animals in each group. In the stimu-

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lated group, eight mice were used for two-cell-stage experiments (23.0 ± 13.9 embryos/female) and seven mice for the blastocyst experiments (12.4 ± 6.1 embryos/female). The unstimulated group consisted of nine mice for the two-cell-stage experiments (6.9 ± 2.4 embryos/female) and eight mice for the blastocyst experiments (7.3 ± 5.9 embryos/female). Not all embryos of each female contributed to the results because, in some cases, the incubation of too many embryos would prolong the duration of an experiment, thereby influencing the results. Moreover, some embryos were lost during the procedures.

The Mann-Whitney test was used for statistical evaluation of the results.

RESULTS

The ^{35}S -methionine uptake and incorporation results are presented in Table I. In both the two-cell and the blastocyst stage, incorporation of ^{35}S -methionine into proteins did not differ significantly ($P > 0.05$) if embryos obtained from stimulated and unstimulated females were compared. The uptake of embryos obtained from stimulated females was significantly lower ($P < 0.01$) in both developmental stages than the uptake rates of embryos from unstimulated females.

Stimulation did not affect the qualitative patterns of embryonic protein synthesis as determined by one-dimensional SDS-PAGE in both developmental stages.

Table I. Uptake and Incorporation of ^{35}S -Methionine of Mouse Two-Cell Embryos and Blastocysts, Developed In Vivo in Stimulated and Unstimulated Females (Mean \pm SE)

Embryo type ^a	Number of embryos analyzed	Uptake ($\times 10^3$ cpm/embryo/hr)	Incorporation ($\times 10^3$ cpm/embryo/hour)
2-cell			
Unstim. fem.	55	$15.9 \pm 1.3^*$	0.65 ± 0.07
Stim. fem.	146	$12.5 \pm 0.6^*$	0.48 ± 0.02
Blastocyst			
Unstim. fem.	40	$133 \pm 19^{**}$	16.6 ± 1.2
Stim. fem.	48	$82 \pm 11^{**}$	15.9 ± 1.7

^a Unstim. fem., unstimulated female; stim. fem., stimulated female.

* $P = 0.0013$, Mann-Whitney test.

** $P = 0.0003$ by Mann-Whitney test.

DISCUSSION

The uptake and incorporation of amino acids by oocytes and embryos are supposed to be active processes (3). During the development from the zygote to the blastocyst stage of mouse embryos, uptake and incorporation increase (4-6) and the incorporation correlates with the number of cells of the embryo (7). Uptake of methionine becomes partially sodium dependent if the embryo reaches the compacted morula stage (4). Besides the increase of uptake and incorporation of amino acids during embryo development, there is also an alteration in qualitative protein synthesis. In most mice strains, the embryonic genome is activated during the two-cell stage (6), leading to developmental stage-dependent changes in messenger ribonucleic acid (mRNA) synthesis and subsequent protein synthesis (8). For these reasons, the measurement of uptake and incorporation gives information about the viability of an embryo. Moreover, the evaluation of protein patterns gives information about the nuclear status of an embryo.

Our results indicate that stimulation with hMG and hCG reduces the uptake process. Although the incorporation process (quantitatively and qualitatively) is not influenced significantly, we have to conclude that the mean embryo viability decreases due to stimulation. Observations of Elzamar and colleagues (9) are in agreement with our findings. They described a lower cell number and mitosis index of day 3 mouse embryos of stimulated females. If development of these embryos continued in the biological mother, there was a drop in implantation ratio of 50% compared to the unstimulated situation. On the other hand, if the retarded embryos were transferred to unstimulated recipients, there was no decrease in implantation ratio (9,10). By all events this means that the stimulated uterus is not an optimal site for implantation. This is also known from human oocyte donation programs, which give higher implantation rates than homologous IVF programs (11). The retardation in embryo development might be due to this suboptimal uterine (or tubal) environment too. However, it is also possible that the oocytes/embryos are directly influenced by the stimulation procedure. Both possibilities will be evaluated in future research. Moreover, with our experimental setup we can study whether in vitro culture can rescue embryos of stimulated females. In this perspective it is specially interesting to study

the effects of coculture with different cell types on embryo viability.

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