J. Nuclear Agric. Biol., 17 : 57-60 (1988)

Short Notes

In vitro Incorporation of ^{3}H-Leucine and ^{3}H-Uridine into Endometrial Cells of Goat

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The interaction of uterine cellular components with ovarian progesterone and or oestrogen depends upon the quantity of hormones available to the cells and hormone receptors in the cells. The biochemical events, that occur prior to implantation at the molecular level in the endometrium, have been investigated in a number of species including man. In rabbit, progesterone induces uteroglobin synthesis in the uterus[1], whereas estrogen stimulates the synthesis of a number of nuclear and cytoplasmic proteins in rat uterii[2,3]. The hormone induced alterations in the endometrium are pre-requisites to implantation because these are involved in preparing the endometrium for the implantation of the blastocyst. In the present study, an attempt was made to isolate the endometrial cells from goat; and then to elucidate the effect of different hormones such as progesterone and estradiol-17β, on protein and RNA synthesis in suspension cell culture.

Medium for cell culture was freshly prepared before each experiment. Powdered medium 199 was freshly dissolved in double distilled water and was buffered with $NaHCO_3$ (350 mg/l) and HEPES (4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid) (2.3 g/l). The pH was adjusted to 7.4 with 0.1 N NaOH. Foetal calf serum was made steroid free using Norit A[4]. All the glassware used in the experiment were siliconized with 1 per cent siliclad solution, rinsed twice with glass distilled water and then oven sterilized, Immediately after the preparation, all the solutions were filtered and sterilized by passing through Millipore filters of 0.2 μ pore size.

After slaughter, the intact genital tract was dissected from the doe and was transported to the laboratory under sterile conditions in 150 ml flask containing PBS (Dulbecco's phosphate buffered saline) kept on crushed ice. The uterine horns were separated from the genital tract and were made free of adherent mesentery and adipose tissue. Each horn was slit open along the length of the tract to expose the lumen with the help of scissors. The luminal layer was washed 5 times with PBS to remove mucus secretions. Caranucular and intercaranucular portions of the endometrium were cut from the luminal layer of horns as small pieces with scissor. Small thin pieces of endometrium were washed twice with PBS and were minced until the resulting pieces were less than 1^2 mm. The minced tissue was placed on nylon filter (100 μ mesh size) and was washed with PBS to remove free cellular debris. The minced tissue was disaggregated into isolated cells by minor modification of the method[5] used for the rat uterus. The minced endometrial tissue (6 to 7 g) was transferred in trypsinizing flask containing 60 ml medium 199 supplemented with 0.2 per cent collagenase and 0.01 per cent deoxyribonuclease (pre-warmed to 37°C). The flask was then placed in a incubator at 37°C for a period of 30 min. The contents of the flask were shaken continuously at slow speed. At the end of incubation period, the digested content of the flask was passed through a series of Pasteur

pipettes of decreasing sizes, the ends of the pipettes were fire polished. This procedure released cells from the tissue pieces. The cells were decanted into 20 ml siliconized centrifuge tubes through a funnel lined with a nylon filter and centrifuged at 3000 x g for 10 min. The cell pellet was resuspended in enzyme-free medium containing 10 per cent steroid-free fetal calf serum and antibiotic mixture (pancillin-G, 100 IU mg; streptomycin sulfate, 100 μg/ml). Cells were counted in a blood cell hemocytometer and the viability was determined by trypan blue exclusion dye technique. The cells were placed in primary culture in siliconized glass culture vials (15 $\times$ 75 mm). One ml of medium (medium 199 supplemented with 10 per cent steroid-free fetal calf serum; and pencillin and streptomycin sulfate) containing 20,500 cells in each vial was incubated in a metabolic incubator.

Cells in primary culture were pulse labelled at zero time to ^{3}H-leucine (1.5 μ Ci/ml) or ^{3}H-uridine (1.0 μ Ci/ml) to assess the protein and RNA synthesis, respectively. The 10 μl quantity of each was added per ml of medium containing cells to give the required concentration of the radioisotope. Cycloheximide (25 μg/ml) was used to check the incorporation of ^{3}H-leucine and to assess the metabolic integrity. Cells were exposed to progesterone and estradiol-17-β. The concentrations of these hormones used were: progesterone, 10^{-8} M, 10^{-7} M, 10^{-6} M and estradiol-17 β, 5×10^{-11} M, 10^{-10} M, 10^{-9} M. One μl ethanol containing the hormone was added per ml of medium containing cells to give the required concentrations of the hormone. Equal quantity of ethanol was added to the control. At the end of the incubation, each cell culture vial was centrifuged at 300 $\times$ g for 10 min at 4°C. The cell pellet was washed twice with saline by suspending the pellet each time cold saline and then centrifuging as above. The pellet was suspended in 1.5 ml of cold 7 per cent TCA and left at 0°C for 2 hr. At the end of incubation, each vial was centrifuged at 2000 $\times$ g for 10 min. The pellet was then suspended in 1.5 ml of 5 per cent TCA and centrifuged as above. Finally, the pellet was washed with 5 ml of ethanol and treated with 5 ml of diethyl ether. The pellet was dried and solubilized by adding 1 ml of soluene-350 into each vial. After 30 min, the contents of each vial were transferred into a scintillation vial and 5 ml of scintiallation fluid was used in each vial. The rodioactivity was measured in a liquid scintillation counter (Packard Tricarb Prias Liquid Scintillation Counter-Model BPLD S.No. 00099).

The enzymatic method used in the digestion of goat endometrial tissue resulted in isolated cells with few clumps of 2 to 4 cells. The viability of the cells was 95.6 per cent as judged by trypan blue exclusion dye technique. There was a time-dependent increase of ^{3}H-leucine incorporation into TCA-precipitates of cells in suspension culture for 24 hr (Fig. 1). Cycloheximide blocked the incorporation.

Progesterone and estradiol-17β caused an increase in ^{3}H-leucine incorporation into protein and ^{3}H-uridine incorporation into RNA when endometrial cells in suspension culture were incubated for 12 hour period (Table 1). The increase was 11.28, 18.67 and 10.93 per cent of leucine incorporation over control, observed in the presence of 10^{-6}M, 10^{-7} M and 10^{-8} M progesterone concentrations respectively. With the same progesterone concentrations, the respective increase in ^{3}H-uridine incorporation into cells was 19.37, 4.58 and 9.25 per cent over control. Estradiol-17β led to 19.66, 19.07 and 11.17 per cent increase in ^{3}H-leucine incorporation over control, concentrations being 5×10^{-11}M, 10^{-10}M and 10^{-9}M respectively. The corresponding increase in the ^{3}H-uridine incorporation was 36.91, 20.15 and 47.83 per cent over control with the same respective concentrations.

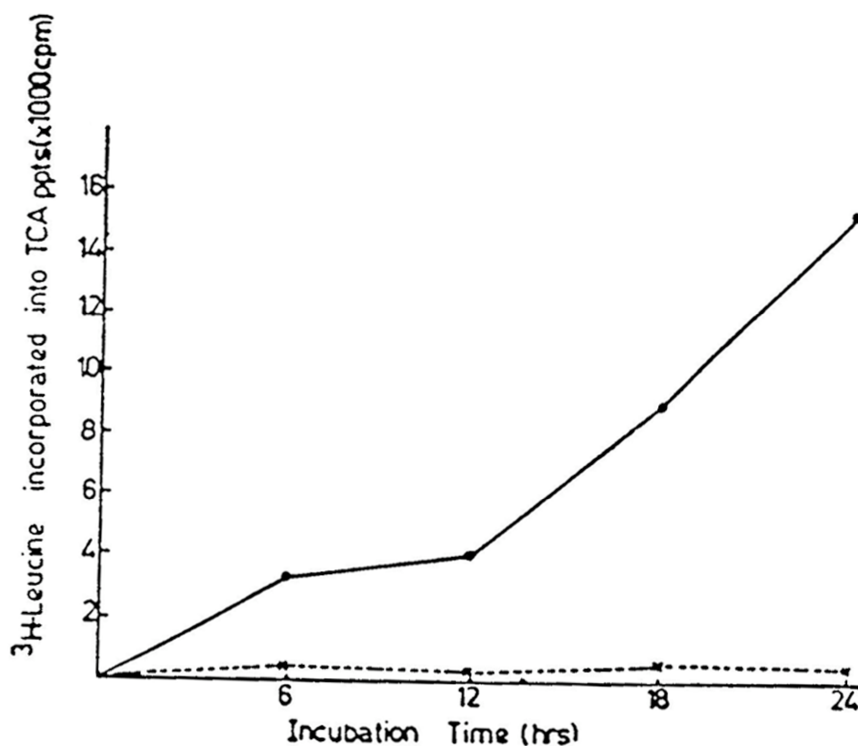

Fig. 1. Incorporation of (^{3}H)-Leucine into TCA-precipitates of endometrial cells (20500 cells/ml at 37°C in the absence (●) and presence (×) of *Cycloheximide*

Table 1. Effect of progesterone and estradiol-17β on the incorporation of ^{3}H-leucine and ^{3}H-uridine into endometrial cells (20,500 cells/ml/culture vial) after 12 hrs in suspension culture

	Conc.	^{3}H-leucine* (cpm)	^{3}H-uridine** (cpm)
Progesterone	10^{-8}M	4743	1510
	10^{-7}M	5058	1722
	10^{-6}M	4728	1332
Estradiol-17β	5×10^{-11}M	5100	1732
	5×10^{-10}M	5075	1520
	5×10^{-9}M	4738	1570
Control		4262	1265

*1.5 μCi/ml, **1.0 μCi/ml

The types and concentrations of the enzymes used for the digestion of endometrial tissue into isolated cells by different workers differed with different species. Rabbit endometrial cells were isolated by using collagenase, hyaluronidase and trypsin[6].

Human endometrium was digested into isolated cells by the use of trypsin, pancreatin collagenase and deoxribonuclease[7]. In the current study, the method used for rat uterine cells was modified by doubling the concentration of collagenase in the enzymatic mixture because of higher content of connective tissue in the caruncles of ruminant endometrium[8,9]. This modified technique provided 95.6 per cent viable cells as suspension culture which were active to synthesize protein as a function of time.

The effect of steroid hormones on uterine metabolism with special reference to protein or RNA synthesis have been demonstrated in different species of immature[10], ovariectomized[11,12] cyclic[13,14] and in pregnant mammals[15,16]. A number of such studies have also been conducted using organ culture[17], explant culture[18], suspension cells[5], cell monolayers[19,20] and established cell lines[21]. The effects have been observed at few minutes as well as at few to several hours of hormonal treatments in the literature. The current study showed the effect of progesterone and estradiol-17β on RNA and protein synthesis in goat endometrial cells in suspension culture at 12 hrs.

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