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Secretory Proteins of the Hamster Cervix, Uterus and Oviduct: The Effects of Estradiol, Progesterone and Testosterone on the Proteins Secreted into the Medium

L. Girija Devi, M. Basheer Ahmad and S. Shivaji*

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

The present study was directed towards identification of proteins synthesized and secreted by the cervix, uterus and oviduct of immature hamsters and by the uterus of ovariectomized adult hamsters. Hamsters were treated with estradiol, progesterone or testosterone for 3 consecutive days after which the tissues were incubated *in vitro* and [^{35}S]methionine labelled proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The results demonstrate a great degree of similarity between the proteins synthesized and secreted by the cervix, uterus and oviduct of hamsters. Treatment of hamsters with estradiol consistently increased the synthesis of a 60 kDa protein in the cervix, uterus and oviduct. Further, estradiol also consistently suppressed the synthesis of a 14, 30 and 72 kDa protein in the uterus but not in the cerivx and oviduct. In the cervix, in addition to the 60 kDa protein estradiol also induced the synthesis of two other proteins (a 38 and 56 kDa protein). Testosterone and progesterone did not induce or suppress the synthesis of the secretory proteins in the hamster cervix, uterus and oviduct. In hamster the 60 kDa protein could serve as a marker of gene expression following hormone action.

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INTRODUCTION

Fertilization in mammals occurs in the fallopian tubes in the female reproductive tract and depends on the gametes and their microenvironments, the male and the female reproductive tracts. Thus, studies directed towards characterization of the chemical components (especially proteins) of the male and the female reproductive tracts may unveil the basic conditions conducive for successful fertilization.

As of now, close to 400 different secretory proteins of the male reproductive tract of mammals have been purified and characterized [1] with respect to their influence on motility of spermatozoa [2, 3] and fertilizing ability of spermatozoa [4–6]. But, comparatively little is known about the secretory proteins of the female reproductive tract of mammals. The uterus [7–14] and the oviducts of mammals [15–20] also secrete a number of proteins. These secretory proteins of the female reproductive tract vary from animal to animal, from region to region and also in their quantity depending on the hormonal status of the animal. Further, the uterine and oviductal secretory proteins have different functions and are known to influence spermatozoal motility, capacitation, acrosome reaction, fertilizing ability, implantation and early development of the embryo [17, 21, 22]. Additionally, such proteins may also play a significant role in immunity and exhibit bactericidal activity [11] and maintain a sterile uterine environment. In addition to the uterine and oviductal secretory proteins, the proteins which are secreted by the cervix all through the menstrual cycle in women also influence sperm-migration [23].

In this paper, we have monitored the synthesis of secretory proteins by the female reproductive tract (cervix, uterus and oviduct separately) of immature hamsters and adult ovarectomized hamsters injected with either estradiol, progesterone or testosterone. The approach was to incubate the explants (cervix, uterus or oviduct) in serum-free medium with a radioactive precursor ([^{35}S]methionine) and analyze the secretory proteins by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by fluorography.

*Correspondence to S. Shivaji.
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MATERIALS AND METHODS

Animals

Golden hamsters (*Mesocricetus auratus*) were reared at constant temperature (22 ± 1°C) and light (6 a.m. to 6 p.m.) and were fed and watered *ad libitum*. The immature animals were 20 days old and the mature animals were 6 to 8 weeks old. Only those mature animals which exhibited the invariant 4-day estrous cycle as judged by regular vaginal smears were used. Mature female hamsters which were anesthetized with thiopentone (5 mg per 100 g body wt) and whose ovaries were surgically removed and allowed to recuperate for a period of 3 weeks were used as the ovariectomized (OVX) animals.

Hormone injections and tissue weights

Female immature or OVX animals (a minimum of 10 animals per group) were injected subcutaneously for 3 consecutive days with estradiol (1 to 3 μg per 10 g body wt per day), testosterone (200 μg per 10 g body wt per day) or progesterone (200 μg per 10 g body wt per day) and were killed and used 24 h after the last injection. The control animals received an equal volume of 1,2-propane diol, the vehicle in which the above steroids was dissolved. The weights of the cervix, uterine horns and the oviducts was recorded separately for each animal.

In vitro incubations

Hamsters were killed by decapitation, and the cervix, uteri and oviducts were dissected free of mesentary and fat, excised and transferred to methionine-free MEM (minimal essential medium) at 5°C. The cervix and oviducts were separated, blotted, weighed and put into fresh medium. The two uterine horns were then slit longitudinally, rinsed in fresh medium twice, blotted, weighed and transferred to fresh, methionine-free MEM. The two uterine horns from each immature animal were incubated together in 1 ml of methionine-free MEM containing [^{35}S]methionine (50 μCi/ml) supplemented with streptomycin (1 μg/ml) and penicillin (100 U/ml) for 6 h at 37°C in a CO_2 incubator in the presence of 95% air and 5% CO_2 and continuous shaking. Since 10 animals were used for each treatment group the incubation of the uterine horns was conveniently carried out in a 24 well tissue culture plate. The uterine horns from adult animals of each group were incubated (1 g tissue per 4 ml of medium) in a 35 mm plastic sterile petri plate. Oviducts and cervix from each treatment group ($n = 10$ animals) were pooled together and incubated in MEM (1 g tissue per 4 ml of medium) as above in 35 mm petri plates. After 6 h of incubation, the radioactive medium was collected with a micropipette and centrifuged at 10,000*g* for 30 min to pellet the debris. The supernatant was frozen immediately in liquid nitrogen and stored at −70°C until required. In a separate experiment cervix, uterus and oviduct were incubated in MEM as described above and at regular intervals of time samples of the medium (25 μl) were recovered in triplicate and [^{35}S]methionine incorporation into secretory proteins was determined.

Estimation of [^{35}S]methionine incorporation

Hot TCA (trichloracetic acid) precipiation method [24] was used to estimate the incorporation of [^{35}S]methionine into the secretory proteins in duplicate aliquots (25 μl) of the medium. Radioactivity was determined using a Packard Tricarb liquid scintillation counter using a toluene based scintillation fluid. The protein content of the media was determined using the method of Lowry *et al.* [25] with bovine serum albumin (BSA) as the standard.

SDS–PAGE

SDS–PAGE was carried out according to the procedure of Laemmli [26]. Aliquots of the medium containing equal counts per minute were analyzed either on a 7.5 or 10% SDS-polyacrylamide gel. After the completion of electrophoresis the gel was stained with 0.08% Coomassie Brilliant Blue R-250 in methanol–acetic acid–water (50:7:43, by vol) and destained in the above solvent mixture without the dye. All the samples were electrophoresed separately.

Table 1. Estradiol induced increase in the wet weight of uterus, oviduct and cervix of immature and adult OVX female hamsters[a]

Animals	Steriod (μg/10 g body wt)	Uterus (mg)	Oviduct (mg)	Cervix (mg)
Immature control	—	27 ± 4	10 ± 1	6 ± 2
Immature estradiol-treated	0.5	52 ± 2	12 ± 1	10 ± 1
	1	87 ± 6	14 ± 1	12 ± 2
	2	122 ± 12	16 ± 1	16 ± 4
Immature progesterone-treated	200	27 ± 2	9 ± 1	7 ± 2
Immature testosterone-treated	200	23 ± 2	9 ± 2	6 ± 2
Adult OVX control	—	79 ± 15	—	7 ± 1
Adult OVX estradiol-treated	2	262 ± 50	—	25 ± 4

[a]Animals were injected for 3 consecutive days with the respective dose of the steriod per day. A minimum of 10 animals were used in each group.

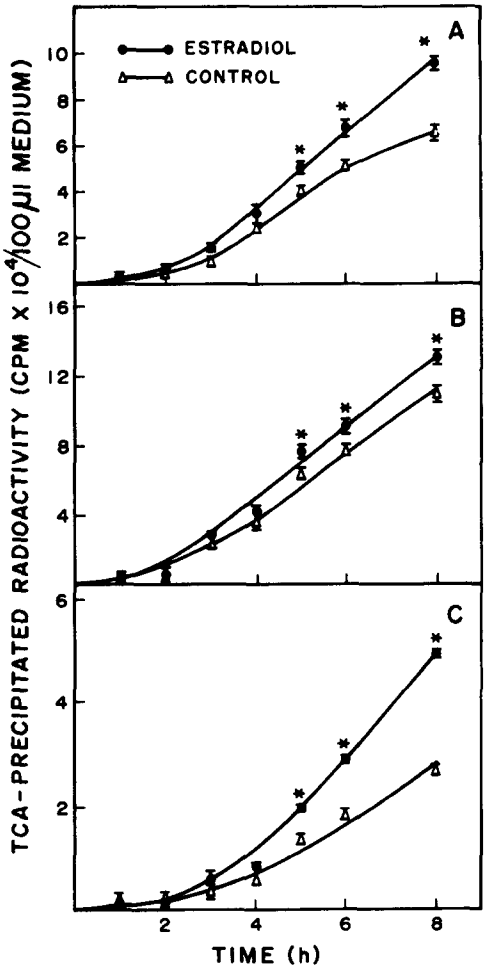

Fig. 1. Time course of [^{35}S]methionine incorporation into proteins synthesized and secreted by the uterus (A), oviduct (B) and cervix (C) of control and estradiol-treated immature hamsters. The estradiol-treated animals were injected subcutaneously with estradiol (2µg per 10 g body wt per day) for 3 consecutive days. The control animals were injected an equal volume of 1,2-propanediol, the vehicle. At the required time points aliquots of the medium in triplicate were precipitated with TCA and the radioactivity was determined. The results are represented as the mean ± SEM and the asterisks indicate significant difference at the 0.05 confidence level as determined by the Student's *t*-test.

Autoradiography of the gels

The gels following fixation of proteins were washed thrice with distilled water (10 min each change) and then immersed in an autoradiography enhancer (from NEN Research Products, MA, 02118) and incubated for 30 min at room temperature with continuous shaking. The gel was then washed a few times with distilled water and allowed to remain in water for 15 min until the fluorophore precipitated so as to make the gel uniformly white. The gel was then dried and exposed to Kodak X-Ray film at −70°C for 3 to 4 weeks. The fluorograms were scanned using a Molecular Dynamics laser densitometer. All the autoradiograms of several gels and each experiment was repeated at least three times.

RESULTS

Manifestation of tissue-response to steroids

Immature female hamsters following treatment with estradiol exhibited an increase in the weight of the uterus, oviduct and cervix (Table 1). But, animals treated with progesterone and testosterone did not show any significant change in the weight of the cervix,

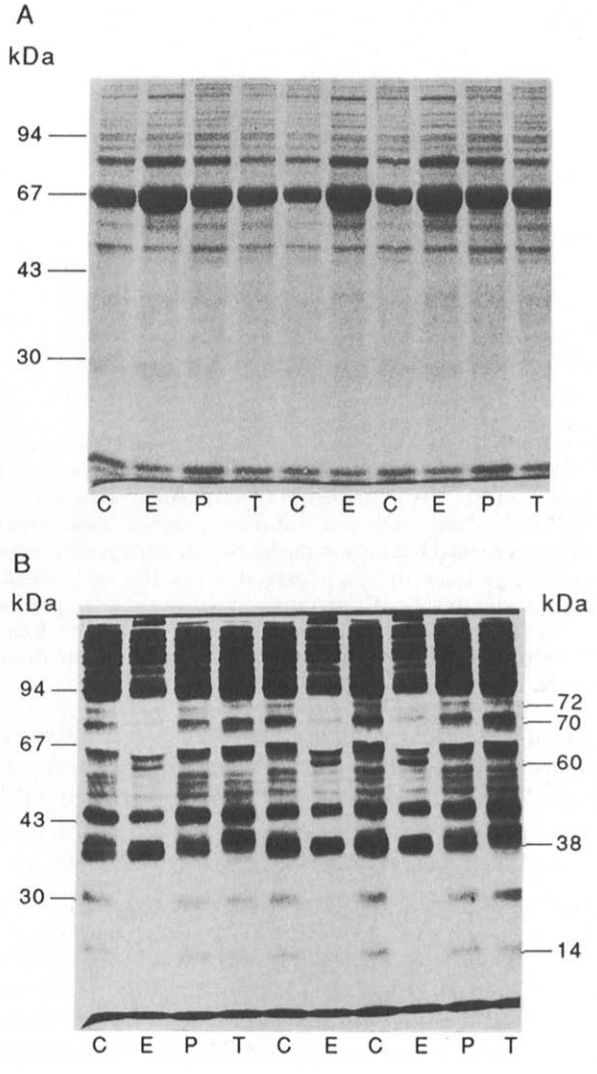

Fig. 2.(A and B). Comparison of the protein profiles (A) and the radiolabeled secretory proteins (B) of the immature hamster uterus from control (C), estradiol (E), progesterone (P) and testosterone (T) treated immature hamsters following analysis by SDS–PAGE (10% gel) and autoradiography. Animals were injected with E, P and T at a dose of 2, 200 and 200 µg, respectively per 10 g body wt per day for 3 consecutive days. Each lane was loaded with an equal amount of TCA precipitable radioactive proteins (100,000 cpm). (B) is an autoradiogram of (A). The marker proteins in this and all other gels were phosphorylase B (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa) and carbonic anhydrase (30 kDa). Each lane represents tissue obtained from a different animal.

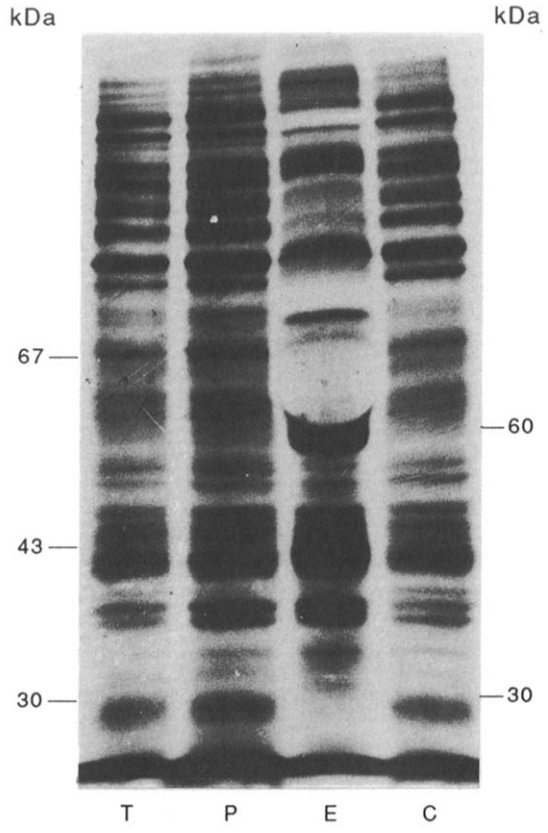

Fig. 3. Radiolabeled secretory proteins of uteri from control (C), estradiol (E), progesterone (P) or testosterone (T) treated adult OVX hamsters following analysis by SDS–PAGE (7.5% gel) and autoradiography. The control animals were injected only with the vehicle whereas the other animals were injected 2 μg of estradiol per 10 g body wt (E), 1 mg of progesterone (P) or 1 mg of testosterone (T) per day for 3 consecutive days. Each lane was loaded with 100,000 cpm of TCA precipitable radioactive protein from the medium.

uterus and oviduct compared to the control untreated animals (Table 1). Further, the weight of the cervix and uterus (Table 1) in the adult estradiol-treated OVX female was also greater than that observed in the OVX female which was injected only with the vehicle.

In vitro protein synthesis

The synthesis and secretion of proteins by immature hamster uterus [Fig. 1(A),] oviduct [Fig. 1(B)] and cervix [Fig. 1(C)] was initially very slow (up to 2 h) but subsequently it increased sharply and this continued till 8 h. It was invariably observed that incorporation of [^{35}S]methionine into secretory proteins of the cervix, uterus and oviduct was considerably greater in the estradiol-treated immature hamsters [Fig. 1(A–C)]. All experiments were repeated 3 to 5 times and the increase in incorporation of [^{35}S]methionine into secretory proteins in the presence of estradiol was consistently higher compared to the control animals. Further, the increase in incorporation at 5, 6 and 8 h was also observed to be significantly different at the 0.05 confidence level as determined by the Student's t-test. In estradiol-treated OVX hamsters also the uterine horns secreted more [^{35}S]methionine labeled proteins into the medium ($5.95 \pm 0.23 \times 10^4$ cpm per 100 μl of medium) than the respective control animals ($2.6 \pm 0.21 \times 10^4$ cpm per 100 μl of medium).

Secretory proteins of the immature hamster uterus

Preliminary experiments had indicated that the secretory protein pattern of the hamster cervix, uterus and oviduct did not depend on whether the tissues were incubated for 6 or 8 h. Hence, all incubations were carried out for 6 h. The proteins secreted by the immature hamster uterine horns resolved into about 35 distinct bands following SDS–PAGE and Coomassie blue staining [Fig. 2(A)]. However, autoradiography of the above gel indicated that the uterine horns synthesized and secreted a number of proteins which were consistently observed [Fig. 2(B)] in the control, estradiol, progesterone and testosterone-treated immature animals such as the prominent 38 and 43 kDa proteins in all the treatments. The uterine horns from the estradiol-treated immature animal differed in that they showed an increase in the synthesis of a 60 kDa protein and a distinct decrease in the synthesis of a protein of 30 kDa [Fig. 2(B)] compared to the control, testosterone or progesterone-treated animals. Further, in the estradiol-treated animals, proteins of approximate molecular weights 14, 70 and 72 kDa were also suppressed to varying degrees. In the high molecular weight region (>94 kDa) the gels were packed with many radioactively labeled proteins and the intensity of radioactivity of these proteins in the range of 94 to 109 kDa in the estradiol treated uteri were slightly less [Fig. 2(B)].

Secretory proteins of the uterus of OVX hamsters

The uterus of the estradiol-treated OVX hamsters also showed an increase in the synthesis of the 60 kDa protein and suppression of the 30 and 70 kDa proteins, respectively (Fig. 3) as observed in the estradiol-treated immature hamster. In addition at least 3 other proteins above the 72 kDa band were also suppressed. But OVX hamsters treated with progesterone or testosterone had much lower amounts of 60 kDa and a prominent 30 kDa band as in the controls.

Secretory proteins of the immature hamster cervix and oviduct

The oviduct and cervix of immature hamsters also synthesized and secreted a number of proteins into the medium (Figs 4 and 5). Both the tissues showed an induction in the synthesis of the 60 kDa protein in the presence of estradiol. But, unlike the uterus the synthesis of the 30 kDa protein was not suppressed by any particular hormone. Further, two other proteins of M_w 38,000 and 56,000, respectively also showed increased synthesis in the presence of estradiol in the cervix but not in the oviduct (Fig. 5). Testosterone or progesterone did not induce or suppress the synthesis of any

secretory protein by the cervix [Fig. 4(B)] or oviduct (data not shown).

Quantitation of the 30 and 60 kDa proteins

In order to quantitate the above secretory proteins of the female reproductive tract of hamster the intensities of the corresponding protein bands in the various autoradiograms was determined by densitometry of gels in which equal TCA precipitated counts were loaded (Table 2). The data confirm the increase synthesis of the 60 kDa protein in the cervix, uterus and oviduct and a decrease in the synthesis of the 30 kDa protein only in the uterine horns under estradiol influence.

DISCUSSION

The present results confirm an earlier study that had demonstrated a consitent increase in the uterine weight of hamster [27] under the influence of estradiol and also demonstrate that testosterone and progesterone do not induce any increase in uterine weight. The increase in the weight of the cervix, uterus and oviduct in the adult estradiol-treated OVX female and in immature animals injected with estradiol are probably a manifestation of the various biochemical changes that occur in these tissues such as increase in the biosynthesis of proteins and imbibition of water.

Secretory proteins of the uterus

It was observed that estradiol induced the synthesis of a 60 kDa protein in the uterine horns of immature hamsters. This protein with a M_w of 60,000 was detectable even after the medium was treated with Blue Sepharose [7] to remove hamster serum albumin (non-radioactive). Uterine secretory proteins whose syntheses are regulated by estradiol have been described in several mammals such as in rat [8, 9], mouse [10, 28], rabbit [29], guinea pig [30, 31], sheep [32], cat [33], baboon [13] and in human beings [14]. However, very few estradiol-induced proteins were amenable to a detailed characterization probably due to the minute amounts in which these proteins were secreted. The major estradiol-induced protein in the uterus of rat was (structurally related to) complement component C3 [9, 34], in mouse it was lactotransferrin [10, 28] and in human beings many estradiol-induced proteins have been identified (for review see [14]). The estradiol-induced 60 kDa protein secreted by the hamster uterus is different from the above reported proteins with respect to its molecular weight, but it is close to that of the 65 kDa protein in rat [8] and the 57 kDa protein in sheep [32]. Further, like other estradiol-induced proteins which were not induced by testosterone or progesterone as in rat [8], sheep [32], baboon [13], guinea pig [30, 31] and dog [12] the 60 kDa protein of hamster was also not induced thus confirming that its induction is estradiol-specific. That the induction is indeed estradiol specific is further confirmed by the observations

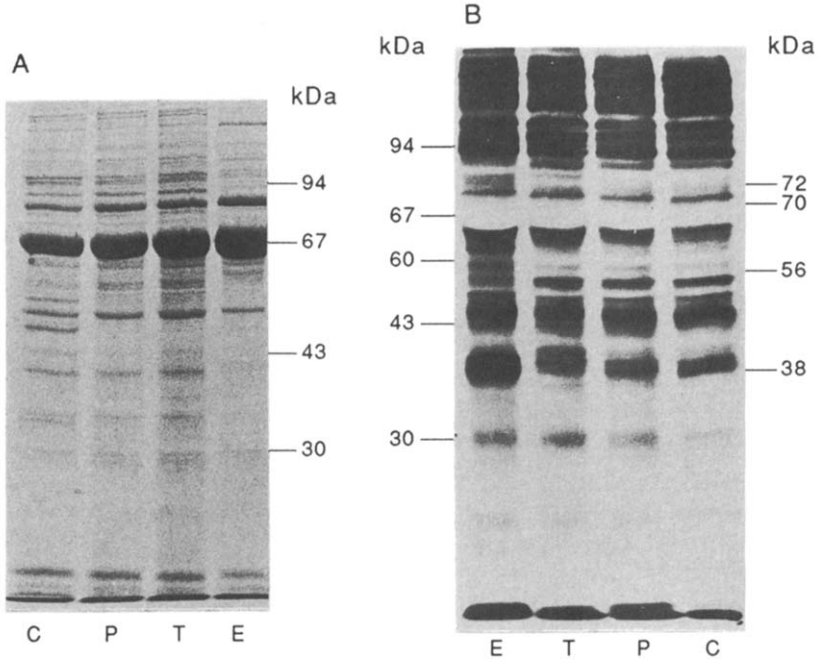

Fig. 4. Comparison of the protein profiles (A) and the radiolabeled secretory proteins (B) of the immature hamster cervix from control (C), estradiol (E), progesterone (P) and testosterone (T) treated immature hamsters following analysis by SDS–PAGE (10% gel) and autoradiography. Animals were injected with E, P and T at a dose of 2, 200 and 200 μg, respectively per 10 g body wt per day for 3 consecutive days. Each lane was loaded with an equal amount of TCA precipitable radioactive proteins (100,000 cpm) from the medium. (B) is an autoradiogram of the gel shown in (A).

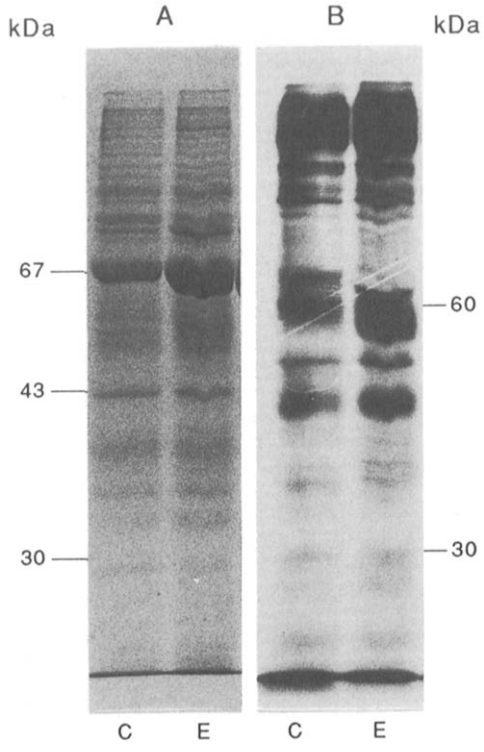

Fig. 5. Radiolabeled secretory proteins of oviducts of control (C) and estradiol-treated (E) immature hamsters following analysis by SDS–PAGE (10% gel) and autoradiography. The control animals were injected only with the vehicle whereas the other animals were injected with 2 μg of estradiol per 10 g body wt per day for 3 consecutive days. Each lane was loaded with 10,000 cpm of TCA precipitable radioactive protein from the medium. Lanes C and E in (B) are the autoradiograms of the respective lanes in (A) which were stained with Coomassie blue.

that it is induced in estradiol treated adult OVX hamsters and in adult hamsters in the estrus stage (data not shown).

In hamster, estradiol was also observed to suppress the synthesis and secretion by the uterus of at least three proteins (14, 30 and 72 kDa) and a few high molecular weight proteins. These proteins were normally present in control animals and in animals treated either with progesterone or testosterone. An acidic protein cP1 (M_w 37,000) in dog uterus [12] and a 40,000 M_w protein in mouse [35] were also shown to be suppressed by estradiol. Further unlike in guinea pig [31], rabbit [29], sheep [32], pig [11], baboon [13] and man [14], progesterone did not induce the synthesis of any secretory protein in the uterus of the hamster.

Secretory proteins of the cervix

The efficiency of sperm transport from the vagina to the oviduct is dependent on the nature of the mucus secreted by the cervix the consistency of which varies depending on the levels of estradiol and progesterone [36]. Despite its important role studies directed towards characterizing the secretory proteins of the cervix are lacking. In hamster, the cervix i.e. the region extending from the junction of the uterine horns up to the vagina secreted a number of proteins and were similar to that secreted by the uterine horns. In the cervix the 60 kDa protein was induced in the presence of estradiol in addition to two other proteins (38 and 56 kDa respectively). Further, the 30 kDa protein was not suppressed by estradiol. In rat the proteins secreted by the uterine horns and cervix following estradiol treatment were identical [8] though differences were evident in hamster.

Secretory proteins of the oviduct

The hamster oviduct also secretes a number of proteins but the 60 kDa protein (as in the uterus and cervix) was the only protein which was induced by estradiol. Robitaille *et al.* [37], in their study on hamster oviducts, did not investigate for the presence of secretory proteins with respect to various hormone treatments. Hence, a comparison of the present results would not be correct. However, their results did indicate a prominent 43 kDa protein and a protein with a molecular mass of 160 to 250 kDa which was termed 'oviductin' [38]. A number of proteins in the high M_w range (>115 kDa) have also been detected in the hamster (present study), sheep [39], pig [16] and baboon [19]. Apart from estradiol-induced proteins the oviduct of mammals are also known to secrete proteins

Table 2. Quantitation of the 30 and 60 kDa secretory proteins of the immature hamster[a] uterus, oviduct and cervix by densitometric scanning of autoradiograms

	60 kDa protein[b]		30 kDa protein[b]	
Tissue	Control	Estradiol-treated	Control	Estradiol-treated
Uterus	15 ± 6	82 ± 10	53 ± 8	10 ± 3
Oviduct	9 ± 2	20 ± 3	3 ± 1	2.5 ± 1
Cervix	27 ± 3	66 ± 5	38 ± 7	41 ± 7

[a]Immature female hamsters were injected with estradiol for 3 consecutive days and the control animals were injected only with the vehicle for the same period of time.

[b]The figures ± standard deviation (arbitrary units) correspond to the relative areas of the respective protein band in the autoradiograms from a typical experiment.

which are estradiol-suppressed such as the 46 kDa protein in sheep [39] or progesterone induced such as the 60 kDa protein in sheep [39]. However, in the hamster none of the secretory proteins of the oviduct were estradiol-suppressed. The exact function of oviductal secretory proteins is still unknown but they are likely to be involved in various functions of the gametes such as the acrosome reaction [40] of spermatozoa and in gamete interaction [17, 37, 40] and early development.

In summary, the cervix, uterus and oviduct of hamster synthesize and secrete a number of proteins. In all the three regions a 60 kDa protein was consistently induced under the influence of estradiol. Further, estradiol suppressed the synthesis of three proteins (14, 30 and 72 kDa) in the uterus. In the cervix, secretory proteins of the above molecular weights were not suppressed but two proteins (38 and 56 kDa) were induced in the presence of estradiol. Since the 60 kDa protein is specifically induced by estradiol all along the female reproductive tract it would be worthwhile to isolate, purify and characterize this protein with respect to its influence on gamete function.

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