Mechanism of ovarian disruption by neonatal DES exposure: A modified ectopic approach.

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Introduction

Diethylstilbestrol (DES) is the classic example of an endocrine disruptor and exemplifies the damage done by xenoestrogens. DES is a non-steroidal, synthetic estrogen that was developed to supplement a woman's natural estrogen production. DES was first prescribed by physicians in 1938 for women who experienced miscarriages or premature deliveries that were believed due to insufficient levels of estrogen. However, in 1971 Herbst et al. reported the occurance of vaginal clear cell adenocarcinoma in a group of young women who were exposed to DES in utero [1].

We use Syrian golden hamsters (*Mesocricetus auratus*) to investigate the phenomenon of neonatal DES-induced endocrine disruption. Preliminary studies conducted in our lab showed that neonatal exposure to DES induced morphological and functional abnormalities in the hamster ovary.

In order to further investigate the neonatal DES-effects on the immature hamster ovary we exploited the cheek pouch of the Syrian golden hamster. Data collected from prior studies involving cheek pouch transplantations showed that transplanation of prepubertal ovaries while still encapsulated within the bursa hindered the process of vascularization which in turn led to the inability of the cheek pouch to sustain the implant. Our modified approach involves the dissection of ovaries out of the encapsulating bursa from control and neonatally DES-exposed donor hamsters and then transplantation of the naked ovaries into the cheek pouches of control and neonatally DES-exposed host hamsters.

The hamster cheek pouch

The hamster cheek pouch is a convenient and well established site for tissue transplantation studies. It is a diverticular, bilateral, symmetric, and double-walled epithelial structure that is derived from the oral cavity and is used to store and transport food. The pouches expand dorsally from the mouth to the shoulder region located ventral to the ears [2,3]

The hamster cheek pouch has been used extensively due to its ability to accept a variety of grafts [4] because of its "immunologically privileged" nature [5]. No lymphatic tissue exists in the wall of the cheek pouch [6] so the reduced number of Langerhans cells and lack of lymphatic drainage result in blockage of the immune response and thus local immunological tolerance. The areolar connective tissue barrier also aids in cheek pouch immunity [2,5].

In addition to the immunologically privileged nature of the hamster check pouch, other advantages include: 1) The ability to frequently evert the check pouch in order to observe, measure, or photograph implants at different stages without causing any undue trauma to the animal, 2) the membrane is almost transparent, a feature that facilitates the observation of the implant as well as any neovascularization as a result of angiogenesis around it, and 3) implantation of grafts as well as preparation of the cheek pouch is relatively easy and sophisticated or sterilized equipment is unnecessary [4].

Transplantation of ovaries into the cheek pouch

The sex of Syrian golden hamsters was determined within 6-8 hours of birth (day 0) and litter size was adjusted to eight neonates per/litter by eliminating extra males. All animals in a litter were treated with a single subcutaneous injection of 50µl of corn oil vehicle (control hamsters) or vehicle containing 100µg DES (~33 mg/kg body weight). Prior to puberty (day 21), control and DES animals were bilaterally ovariectomized through two dorsal incisions. For this surgical

procedure, animals were anesthetized with an intra-peritoneal injection of Nembutal sodium solution at a dose of 0.15ml/100g of body weight. Excised ovarian masses were then placed in sterile cell culture media prior to implantation.With the aid of a dissecting scope each ovary was dissected out of its bursa and accompanying oviduct.

The transplantation procedure took place while the animal was still under anesthesia. The cheek pouch was everted with forceps and spread on a paraffin plate and held in place with pins. Using a dissecting microscope, an incicison was made with a pair of scissors in the epithelium of the pouch and a pocket was opened between the two epithelial layers using forceps. The ovarian mass was then inserted into the pocket and the inscision was sealed using liquid suture material.

Cheek pouches were inspected weekly by anesthetising the animal, everting the cheek pouch, and measuring dimensions of the ovarian masses. The estrus cycle of each animal was also checked on a weekly basis (an indicator of ovarian function).

At two months of age, the animals were terminated. Viable transplant masses and host uteri were harvested and subsequently fixed in neutral-buffered 4% paraformaldehyde.

Results

Inspections of the everted cheek pouch containing the bursa-free ovarian transplants revealed extensive vascularization. Tissue harvesting occurred two months later and uteri in the ovariectomized and successfully transplanted host hamsters had dimensions similar to those of normal uteri in intact animals (Fig.1) as well as the charactersitc dimensions seen in DES-exposed uteri (Fig. 2). The uteri of the hamsters with unsuccessful ovarian transplants were atrophic due to the absence of ovarian estrogens (Fig. 3).

Conclusions

Our preliminary results support the feasibility of the transplantation procedure and shows that the immature ovary can reach mature functionality once transplanted into the cheek pouch. The ability to perform such transplants allow us to further examine the effects DES has on the immature hamster ovary.

Acknowledgements

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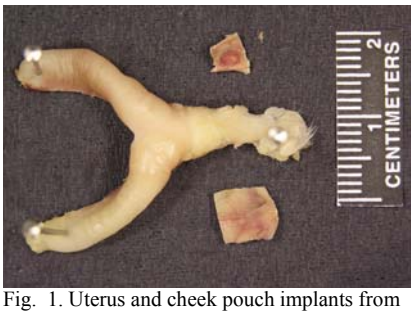

Fig. 1. Uterus and cheek pouch implants from a control homo-transplant

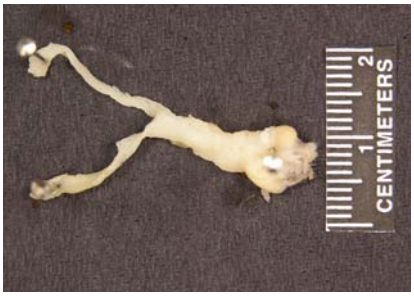

Fig. 2. Uterus and cheek pouch implants from a DES homo-transplant

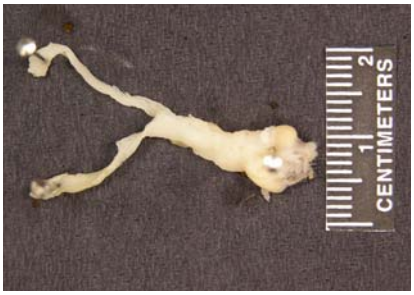

Fig. 3. Uterus with unsucessful cheek pouch implants

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