but surprising increase in the count of epididymo-deferential spermatozoa ($P = 0.09$) which could be related to a secretion or absorption defect of the luminal fluid.

Conclusion: These preliminary results suggested a testicular and genital toxicity of BBP and *p,p'*-DDE in mice exposed *in utero*, as was previously shown in rat. Our results indicated also a toxic effect of both xenohormones in mice exposed during puberty, notably a deleterious effect on the DNA of germ cells for BBP. However, increasing the number of experiments is warranted to confirm all the toxic effects observed.

Reproductive Endocrinology 3

Tuesday 29 June 1999
Room 04-Hall 4+5

14.00–14.15

O-155. Insulin-like growth factor-II (IGF-II) mediates the steroidogenic and growth-promoting actions of follicle-stimulating hormone on human ovarian pre-antral follicles cultured *in vitro*

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FSH is important for ovarian antral follicle growth and steroidogenesis, processes in the human that are believed to be mediated by IGF-II. The objective of this study was to determine if human ovarian pre-antral follicles are also FSH and IGF-II responsive, since the clinical questions and mechanisms underlying the effects on the pre-antral follicle pool of exogenously administered gonadotrophins for fertility therapy and elevated endogenous gonadotrophins in the peri-menopause remain unanswered.

Class 2 pre-antral follicles were isolated from human pre-menopausal ovaries ($n = 6$) and cultured *in vitro* with androstenedione and either no additives or with FSH or IGF-II. FSH (100 ng/ml) stimulated oestradiol production by 3.58 ± 0.4-fold over 48 h, compared to controls without FSH. This effect was completely inhibited in the presence of the IGF-II antagonist, IGF binding protein-4 (IGFBP-4). IGF-II also stimulated oestradiol production by pre-antral follicles with doses as low as 1 ng/ml and within 24 h of treatment. Maximal response of three- to ninefold above control was achieved with 100 ng/ml of IGF-II between 96 and 120 h of culture. IGFBP-4 completely inhibited oestradiol production to basal levels. FSH stimulated IGF-II mRNA in pre-antral follicles about fourfold, determined by RT-PCR. FSH also stimulated follicle growth, determined by light microscopy, 50–68% over 48 h, compared to controls ($P < 0.001$), a process that was inhibited in the presence of IGFBP-4.

Cumulatively, these data demonstrate that FSH and IGF-II stimulate pre-antral follicle oestradiol production, FSH stimulates pre-antral follicle growth and IGF-II rnRNA expression, and IGFBP4 inhibits FSH and IGF-II effects on the pre-antral follicle, supporting IGF-II as a mediator of FSH action on human pre-antral follicles.

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14.15–14.30

O-156. Recombinant human gonadotrophins improve the viability of grafted ovarian tissue

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Introduction: Ovarian tissue can be collected and cryopreserved for women at risk of losing ovarian function. Studies in animals show that frozen–thawed ovarian tissue can restore fertility when surgically returned to the donor, or other histocompatible recipient. Follicle survival within a graft will depend on the graft site. Pieces grafted to well-vascularized sites, such as the kidney, exhibit high survival rates, while grafts to sites such as the abdominal wall have fewer surviving follicles. It is thought that this effect depends on the rate of vascular ingrowth into the graft. Vascular endothelial growth factor (VEGF) is a very potent angiogenic factor. VEGF mRNA levels increase significantly in grafted ovarian tissue and ovaries of mice and rats treated with gonadotrophins. This study examined the hypothesis that the administration of exogenous gonadotrophins will influence follicular survival in ovarian tissue pieces transplanted to sub-optimal sites.

Materials and methods: Immature inbred Balb/C mice were used as donors and recipients. The hormonal treatment regimen comprised twice daily intraperitoneal (i.p.) injections of recombinant human follicle stimulating hormone (3 IU rhFSH, Gonal-F; Serono, Switzerland) and luteinizing hormone (3 IU rhLH, Lhadi; Serono, Switzerland). At surgery recipients were bilaterally ovariectomized and then had a whole ovary inserted into each of two subperitoneal pockets. Six days after surgery all mice were administered 10 IU pregnant mare serum gonadotrophin (Intervet, Australia) i.p to induce follicular maturation, i.e. to test the functional capacity of the transplanted tissue. Seven days after surgery all the grafted ovaries were collected and processed for serial sections, staining and counting of all growing follicles. Six groups were investigated: controls without stimulation (group i); females given a 4 day course of rhFSH + rhLH starting before (ii), during (iii) or after (iv) insertion of normal ovaries (to examine recipient effects) and normal females receiving ovaries from mice treated with rhFSH + rhLH for either 2 (v) or 4 (vi) days before collection (to examine donor effects).

Results: Compared to the non-stimulated control group the number of growing follicles per ovary increased significantly

($P < 0.005$, analysis of variance) in recipients administered rhFSH + rhLH before and after grafting (group iii), but not if the stimulation stopped or started (groups ii and iv) at the time of surgery. Ovaries collected from donors stimulated for 2 days before collection (group V) showed a tendency to an increased number of growing follicles. If the stimulation lasted 4 days (group vi), the increase was highly significant ($P = 0.007$).

Conclusion: The administration of rhFSH/rhLH can improve the viability of grafted ovarian tissue.

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14.30–14.45

O-157. The gonadotrophin releasing hormone mRNA and protein expression in Vero cells

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Introduction: In an attempt to overcome developmental arrest of early embryos cultured in media alone, various co-culture systems have been developed including Vero cells. We have previously demonstrated that embryos co-cultured with Vero cells display enhanced development *in vitro* and this could be due to an interaction between the embryo and cellular layer mediated by paracrine cytokine (IL-1β). In addition, we have recently shown that both gonadotrophin releasing hormone (GnRH) and its receptor are expressed at the mRNA and protein level *in vivo* by human endometrium throughout the entire menstrual cycle. Moreover, we have also demonstrated that preimplantation embryos (mouse and human) also express this hormone and its receptor, suggesting that GnRH may also play a role in the embryonic/endometrial dialogue during early implantation. Thus, we postulated that Vero cell monolayer used in embryo co-culture may provide an environment similar to endometrial cavity; the embryotrophic effect may be due to paracrine regulation of GnRH by co-cultured cells. Therefore, we assessed GnRH and its receptor mRNA and protein expression in Vero cell monolayer.

Materials and methods: Viable Vero cells (1×10^5/well) were cultured in multiple-well tissue culture plates before the introduction of embryos and 4-well chamber slides for immunohistochemical study containing Dulbecco's modified Eagle's medium (DMEM) with 5% fetal bovine serum. Confluent cell monolayer was then cultured in medium containing human tubal fluid and DMEM in 1:1 ratio until use. Total RNA extracted from Vero cells was reverse transcribed into cDNA and amplified by polymerase chain reaction (PCR) using specific primers for GnRH target cDNA (399 bp). The expression of GnRH receptor (231 bp) was amplified by two-round nested PCR. To determine the presence of GnRH at the protein level in Vero cell monolayer, cells were fixed in chamber slides until processed for immunohistochemical staining using avidin–biotin alkaline phosphatase method. Human luteal phase endometrium was used as a positive control. Deletion of the primary antibody was used as a negative control.

Results: The GnRH and GnRH receptor mRNA were both amplified in Vero cells by PCR. Immunoreactive GnRH at the protein level was present with intense staining in Vero cells monolayer according to avidin–biotin alkaline phosphatase method.

Conclusions: Our results suggest that Vero cell GnRH expression at the mRNA and protein level may play a substantial role in early embryo/epithelial dialogue during embryo co-culture, suggesting the embryotrophic effect may be due to expression of GnRH by co-cultured cells.

14.45–15.00

O-158. Developing new techniques to assess endocrine-disrupting chemical effects on human fetal testis

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Introduction: The incidence of reproductive abnormalities in humans is increasing: falling sperm counts and rising rates of testicular cancer, hypospadias and cryptorchidism. These effects may be attributable to in-utero exposure to endocrine-disrupting chemicals (EDC). We previously described an in-vitro culture system in which the EDC dieldrin inhibits the gonadotrophin-stimulated testosterone and inhibin B secretion [Brown E. *et al.* (1998) *J. Reprod. Fertil.*, Abstract Ser., **21**, 55]. Rodent studies have shown that in-utero exposure to EDC affects specific genes in the developing gonad, such as the steroidogenic enzyme cytochrome *P*450 17α-hydroxylase/C17-20-lyase (*P*450c17) [Majdic G. *et al.* (1996) *Endocrinology*, **137**, 1063–1070]. The androgen receptor (AR) is also thought to be an important target for EDC. We have investigated the expression of these EDC-sensitive genes in 13, 15 and 18–19 week human fetal testis, and we present some preliminary data from an ongoing study.

Materials and methods: Human fetal testes, collected at 13 weeks ($n = 2$), 15 weeks ($n = 1$) and 18–19 weeks ($n = 2$) gestation (spanning the period of Leydig cell hyperplasia), were either fixed immediately in Bouin's for 5.5 h or cut into explants and cultured in Dulbecco's modified Eagle's medium/Ham's F12 with 10% human male serum for periods of 2, 8 and 24 h. Bouin-fixed cultured and control tissues were paraffin embedded for (i) morphological analysis by haematoxylin and eosin staining, (ii) immunolocalization of *P*450c17 and (iii) immunolocalization of the AR. Immunostaining was by standard avidin–biotin–peroxidase detection.

Results: *P*450c17 was localized to the testicular interstitial area at all gestational stages examined. In line with increased numbers of Leydig cells, *P*450c17 immunostaining was more