

# Ex vivo culture of human fetal gonads: Manipulation of meiosis regulation affects testis development

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## Background

Alterations in the timing or expression level of players involved in sex determination and differentiation can cause disorders of sex development, gonadal dysgenesis and germ cell neoplasms later in life. The mitosis-meiosis switch, which is directly induced by retinoic acid (RA) and tightly regulated by several other factors, is one of the first manifestations of female gonadal sex differentiation. We hypothesise that a conflict between meiosis-inhibiting (male pathway) and meiosis-inducing signals (female pathway) due to undervirilisation from the somatic niche is a possible mechanism for disruption of normal fetal testis development resulting in dysgenetic testes.

## Aim

To establish an experimental model that allows studies of germ cell – somatic niche interactions in human fetal gonad cultures and determine effects of manipulating RA-signaling pathways involved in meiosis regulation.

## Methods

### Establishment of *ex vivo* culture model for human fetal gonads

#### Samples and Analysis

- Fetal gonadal tissue was isolated from first trimester legally induced abortions (gestational week 7-11).
- Gonad tissue was immediately fixed (age-matched controls) or were set-up in 'hanging drop' cultures.
- Cultures were maintained for two weeks with or without addition of 1  $\mu$ M retinoic acid (RA).
- Samples were formalin-fixed and protein expression was investigated by immunohistochemistry.

#### Set-up

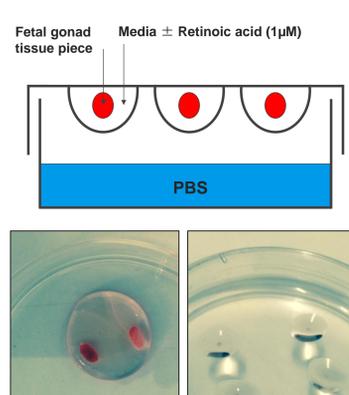


Fig. 1 Overview of the 'Hanging drop' tissue culture set-up.

## Results

### Preserved morphology of human fetal gonads in culture

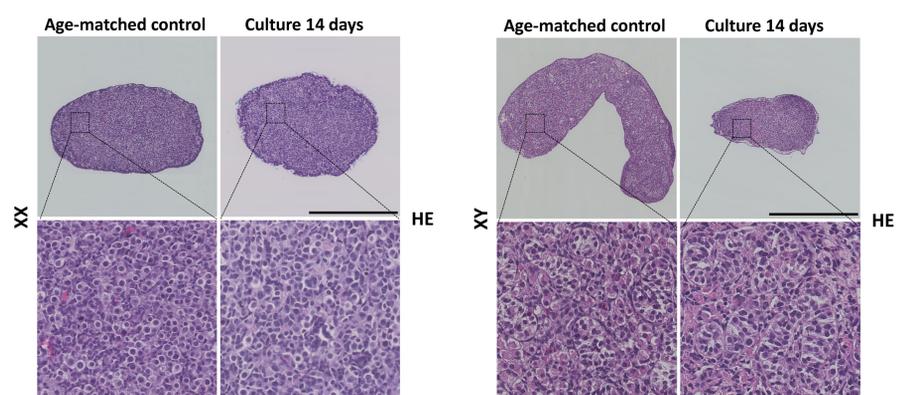


Fig. 2. Morphology of human fetal gonad tissue cultured in 'Hanging drops' for two weeks. Haematoxylin and eosin (HE) staining of human fetal testis and ovary tissue. Fetal gonad tissues (gestational week 7-9) were cultured for two weeks and are compared to not cultured controls age-matched to the end of experiment (GW 9-11). Tissue from 4-7 fetal gonads of each gender was investigated, scale bar: 500  $\mu$ m and 50  $\mu$ m, respectively.

### RA-treatment accelerated initiation of meiosis in fetal ovaries

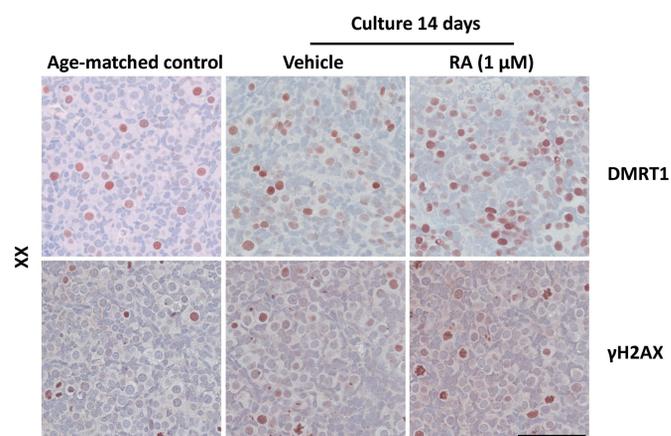


Fig. 3. Expression of the meiosis marker  $\gamma$ H2AX and the meiosis initiator DMRT1 in human fetal ovaries cultured with or without presence of retinoic acid (RA). Cultured samples (GW 7-9) are compared to age-matched controls (not in culture, GW 9-11). Tissue from 4 fetal gonads was investigated. Sections of tissue counterstained with Mayer haematoxylin. Scale bar: 50  $\mu$ m.

### Manipulation of meiosis signaling affects fetal testis development

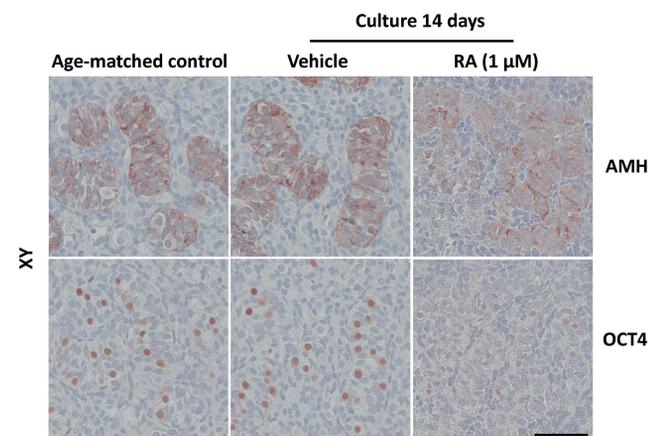


Fig. 4. Expression of the immature Sertoli cell marker AMH and gonocyte marker OCT4 in human fetal testis cultured with or without presence of retinoic acid (RA). Cultured samples (GW 7-9) are compared to age-matched controls (not in culture, GW 9-11). Tissue from 7 fetal gonads was investigated. Serial sections of tissue counterstained with Mayer haematoxylin are shown in each column. Scale bar: 50  $\mu$ m.

### Quantification of RA-treatment effects in fetal gonad cultures

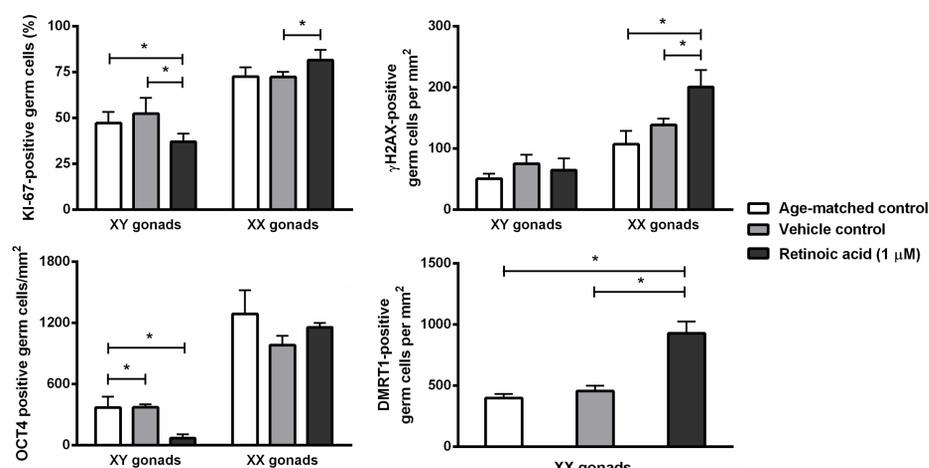


Fig. 5. Quantification of the number of stained cells after RA-treatment for two weeks. Values represent mean  $\pm$  sd of percentage of stained germ cells or number of cells per  $\text{mm}^2$ . \* significant difference ( $P < 0.05$ ).

## Conclusions

### 1. *Ex vivo* culture of human fetal gonads in 'hanging drops' maintained normal development including:

- Normal tissue morphology and level of apoptosis.
- Normal proliferation of germ cells and somatic cells.

### 2. RA-treatment of fetal ovaries accelerated initiation of meiosis:

- Increased the number of meiotic oogonia ( $\gamma$ H2AX-positive).
- Increased number of oogonia initiating meiosis (DMRT1-positive).

### 3. RA-treatment of fetal testes impaired normal testes development resulting in a phenotype that resembles gonadal dysgenesis:

- Reduced expression of immature Sertoli cell marker (AMH).
- Reduced number of gonocytes and proliferating gonocytes.
- Disrupted seminiferous cord structure.

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