

Very early and transient 17 β -estradiol secretion by fetal sheep ovary. *In vitro* study.

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It is generally considered that in mammals, steroidogenesis occurs later in fetal ovary than in fetal testis, which becomes active at the time of sexual differentiation (sheep : Attal, 1969). When the ovary begins to secrete estradiol (and testosterone), oocytes have achieved meiotic prophase, even when steroidogenesis begins before term (bovine : Weniger, Chouraqui and Zeis, 1972 ; macaque : Resko, 1974).

In this report, we re-examine the steroidogenic activity of sheep ovary before and after the beginning of the meiotic process.

Material and methods.

Sheep embryonic gonads of both sexes were removed surgically at 31, 47, and 62-64 days, i.e. before sexual differentiation, before and after onset of meiosis. They were maintained in organotypic culture at + 37 °C for 10 days under the conditions described by Mauleon (1973). The culture medium (TC medium 199, Difco) contained sodium acetate and cholesterol and was supplemented by fetal calf serum, chick embryo extracts or pregnant ewe serum pretreated for 30 min with active charcoal.

The culture medium was changed every two days and stored at - 18 °C. Fresh medium at each renewal served as a blank for the radioimmunoassay.

The unconjugated and free fractions of testosterone and 17 β -estradiol were assayed by RIA after extraction with ethyl acetate cyclohexane. The anti-estradiol antibody (E₂ β -6 CMO BSA) showed no cross reaction with 17 α -estradiol or estrone. The anti-testosterone antiserum (3 CMO BSA) showed a 65 p. 100 cross reaction with 5 α -dihydrotestosterone (Terqui, Dray and Cotta, 1973).

The secretion of 17 β -estradiol and testosterone was estimated by the difference between the amounts found in media after 2-day cultures and those measured in fresh media. The results are expressed in pg/0.5 ml of culture medium in 48 hrs. The significance of the values was tested by analysis of variance.

Under our assay conditions, the quantity of steroid present was sometimes higher than expected. In this preliminary report, the large amounts present are expressed as

values greater than 2 ng/0.5 ml for 17 β -estradiol, and greater than 5 ng/0.5 ml for testosterone.

Results.

The results, obtained after culture of 31 day-old gonads of unknown sex, fell into two groups : those obtained with media containing large amounts of estrogen (> 2 ng), in which presumably female gonads developed, and those achieved with media containing small non-significant quantities of estrogen (38 pg) and small amounts of testosterone (76 pg), significantly higher than the control levels, in which presumably male gonads developed.

*Mean steroid production per 2 days of culture in vitro
by embryonic sheep gonads
(expressed as pg/0.5 ml/48 hrs)*

| Sex | Steroid assayed in medium | Age of embryo (days) at the beginning of the culture | | |
|--------|------------------------------|--|------------------|------------------|
| | | 31 (sex ?) | 47 | 62 |
| Female | 17- β -oestradiol | $> 2\ 000$ * (2) | $> 2\ 000$ * (3) | 16 NS (6) |
| | Testosterone | 30 NS (2) | 32 * (3) | 19 NS (6) |
| Male | 17- β -oestradiol | 38 NS (3) | 23 NS (2) | 58 NS (6) |
| | Testosterone | 76 * (3) | $> 5\ 000$ * (2) | $> 5\ 000$ * (6) |

() : Number of gonad cultures.

NS : non significant.

* : $P < 0.01$.

Large quantities of 17 β -estradiol (means varying from 250 pg to 2 ng/ml) were found in media after culture of 47 day-old ovaries during the entire culture period. Small amounts of testosterone (32 pg), significantly higher than the control media were also detected.

In contrast, older ovaries (62 days) lost this capacity for the production of estradiol or testosterone. This loss of ovarian synthesizing ability may explain why Pomerantz and Nalbandov (1975) were unable to demonstrate the presence of steroids in female sheep embryo at 70 days

Testes from 47 and 62 day-old embryos produce large quantities of testosterone (> 5 ng) and insignificant amounts of estradiol. When co-cultured, testis and ovary from 64 day-old fetuses secreted large quantities of both testosterone (> 5 ng) and 17 β -estradiol (> 2 ng). This suggests that 64 day-old ovary does not lose its ability to aromatize androgens.

Significant steroid values (17 β -estradiol in 31 day-old presumed female gonads and testosterone in those presumed male) progressively increased with each successive 2-day culture.

17 β -estradiol production in 47 day-old ovaries/2 days decreased quickly after 4-6 days. Testis production of testosterone/2 days remained high during the whole culture period.

Conclusion.

Around the time of sexual differentiation (day 31), sheep embryo ovary secretes increasingly large amounts of 17 β -oestradiol in culture. This capacity is lost when the meiotic process is in progress at day 62. The role of this temporary secretion of oestradiol is open to discussion. It is tempting to relate this activity to gonadal sexual differentiation in the female.

In contrast, fetal testis secretes very low quantities of testosterone at the time of sexual differentiation and very high quantities later (47 and 62 day fetuses).

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Résumé. Prélevé pendant la période de la différenciation sexuelle (31^e jour) l'ovaire d'embryon de brebis sécrète de grandes quantités d'œstradiol-17 β en culture.

Cette capacité a disparu à 62 jours, période où les figures de prophase méiotiques sont très nombreuses.

La découverte de cette sécrétion précoce et temporaire d'œstradiol-17 β ouvre des perspectives sur la relation qui pourrait exister entre la présence de ce stéroïde et la différenciation sexuelle.

Au contraire, le testicule produit de très faibles quantités de testostérone à cette même époque, puis de très fortes quantités chez les embryons plus âgés (47 et 62 jours).

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