Steroid secretion by foetal mammal gonads and its regulation by gonadotrophins

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Introduction.

Castration, grafting and substitution experiments performed by Jost (1946-47) in rabbits first demonstrated that the testis controlled male sex differentiation in foetal mammals by a mechanism involving androgen secretion. Later experiments by Jost (1951) suppressing the hypophysis of rabbit foetuses by intrauterine decapitation and giving pregnant mare serum gonadotrophin to the decapitated foetuses showed that this androgen secretion was regulated by pituitary gonadotrophin. These early studies gave no cause to suspect an hormonal function of the ovary, and it was not until 1977 that oestrogen formation by the foetal ovary was established (Milewich *et al.,* 1977).

Since Acevedo *et al.* (1963) first demonstrated by radiochemical methods that the human foetal testis formed testosterone and androstenedione, the secretion of steroids by foetal mammal gonads and its regulation by gonadotrophins have been the subject of numerous investigations. The purpose of the present paper is to review these studies and to stress the points which need particular attention.

A. The testis

1. *Androgen production.*

a. *Effect of LH on testosterone secretion.* — The stimulatory effect of LH on androgen production by the foetal testis *in vitro* — testosterone is the main androgen secreted — has been demonstrated in a great variety of mammalian species (table 1). The foetal testis also responds *in vivo* to injected LH or hCG by increased testosterone secretion. This has been shown in rabbits and rhesus monkeys. Testicular testosterone content and plasma testosterone concentration in the 29-day old rabbit foetus rose 3-fold 30 min after LH was injected into the umbilical vein (Veyssière *et al.,* 1977). One hour after the injection of 100 IU of

hCG into the foetal circulation of the rhesus monkey in late gestation, the serum testosterone level had increased 6-fold (Huhtaniemi et al., 1977b).

b. *Secretion of LH in utero.* — After testis responsiveness to LH *in utero* had been demonstrated, the next logical question was whether the foetal hypophysis secreted LH which could stimulate testosterone secretion.

LH was detectable by radioimmunoassay in the culture media of pituitaries from 13 to 18-week old human foetuses (Groom et al., 1971), from those as young as 7 weeks old (Siler-Khodr et al., 1974) or from those 10 to 19 weeks old (Goodyer et al., 1977). LH was measurable in the pituitary of foetuses as early as 10 weeks of gestation, the youngest stage studied (Hagen and McNeilly, 1975 ; Kaplan and Grumbach, 1976 ; Clements et al., 1976). Immunoreactive LH was first detectable in the serum by 12 weeks of gestation (Reyes et al., 1974 ; Kaplan and Grumbach, 1976 ; Clements et al., 1976 ; Hagen and McNeilly, 1977). The β-LH subunit responsible for biological activity could not be revealed in the pituitary by immunohistochemistry before the 15th week (Bugnon et al., 1977). Taken together, those results indicated that the human foetal pituitary began secreting LH at 10 to 12 weeks.

TABLE 1

Stimulation of testosterone secretion by LH or hCG in the foetal testis of mammals in vitro.

Species	Authors
Human	Abramovich et al. (1974) Ahluwalia et al. (1974) Huhtaniemi et al. (1977a)
Mouse	Weniger and Zeis (1974 and 1975b) Pointis and Mahoudeau (1975, 1977 and 1979)
Rat	Warren et al. (1975) Weniger and Zeis (1975a and b) Picon and Ktorza (1976) Sanyal and Villee (1977) Picon and Gangnerau (1979 and 1980) Paz et al. (1980)
Bovine	Kiser et al. (1975) Shemesh et al. (1978)
Sheep	Mauléon et al. (1977)
Rhesus monkey	Huhtaniemi et al. (1977b)
Rabbit	George et al. (1978) Veyssière et al. (1980b)
Guinea-pig	Brinkmann and van Straalen (1979)
Pig	Raeside and Middleton (1979)

The pituitary of bovine foetuses was found to contain increasing quantities of LH during and after the 3-month stage of gestation, the earliest stage studied, and to synthesize significant amounts of the hormone *in vitro* (Oxender *et al.,* 1972a). No significant difference was observed between the sexes (Workewych and Cheng, 1979). LH was already measurable in the serum of 3-month old foetuses (Oxender *et al.,* 1972b).

LH was measurable in both the pituitary and the serum of foetal lambs from day 55 to term (Foster *et al.,* 1972a, b).

The concentration of LH in guinea pigs from day 50 of gestation, the earliest stage studied, was greater in the foetal plasma than in the maternal circulation. This was proof that the foetal hypophysis secreted LH from this stage (Donovan *et al.,* 1975).

LH was undetectable in the serum of 49 to 80-day old pig foetuses. After that stage, it could be measured and its concentration was substantially higher than in the maternal circulation (Colenbrander *et al.,* 1977).

When both the pituitary and testis of 18-day old mouse foetuses were placed together in organ culture, testosterone release into the medium increased 5-fold (Pointis and Mahoudeau, 1975, 1976). A significant rise in testosterone secretion was also observed with the pituitary and testis of 16-day old foetuses, but not with 14-day old ones (Pointis and Mahoudeau, 1977). However, LH was already detectable in the pituitary at the 14-day stage, but not measurable in the plasma before the 16-day stage (Pointis *et al.,* 1980). Disregarding the slight differences in the results, it can be concluded that LH is first secreted in the mouse foetus between days 14 and 16.

Pituitary LH was first detected in the male rat foetus on day 17 of gestation ; high levels of serum LH had already been found on day 16 (Chowdhury and Steinberger, 1976). The cells containing LH were recognizable from day 18 by immunohistochemistry (Setáló and Nakane, 1976 ; Tougard *et al.,* 1977 ; Watanabe and Daikoku, 1979). So the 17-day stage can be considered as the time when LH secretion begins in the rat foetus.

When both the pituitary and testis of 20-day old rabbit foetuses were put together in organ culture, a 5-fold augmentation in testosterone production was observed (Veyssière *et al.,* 1980a). Nineteen-day old pituitaries, but not 18-day ones, significantly increased testosterone production by 20-day old testes (Veyssière *et al.,* 1980b). LH was first detectable in the pituitary of 19-day old foetuses (Veyssière *et al.,* 1981, 1982). So, it can be concluded that the beginning of LH secretion in the rabbit foetus coincides with the 19-day stage.

Pituitary LH concentrations in foetal ponies increase between days 90 and 150 of pregnancy and then remain constant. BloodᴸLH concentrations are highest between days 100 and 150 (Wesson and Ginther, 1980).

The level of serum LH in male rhesus monkey foetuses remains relatively low throughout gestation, but it is higher than in the maternal circulation (Ellinwood and Resko, 1980).

c. *Autonomous onset of testosterone secretion.* — The question arose whether the onset of testosterone secretion by the foetal testis was dependent on

TABLE 2

Time of onset of testosterone secretion by the foetal testis.

Species	Gestational age	Authors
Mouse	12 days	Weniger and Zeis (1972 and 1975b)
Rat	15 days	Warren *et al.* (1973) Weniger and Zeis (1975a and b) Picon (1976) Feldman and Bloch (1978) Picon *et al.* (1982)
Rabbit	17 to 19 days 18 days	Wilson and Siiteri (1973) George *et al.* (1979)
Human	8 to 10 weeks	Siiteri and Wilson (1974)
Pig	27 days	Raeside and Sigman (1975) Raeside' and Middleton (1979)
Guinea-pig	25 days	Brinkmann (1977)

or independent of LH stimulation. The time of the onset of testosterone secretion by the foetal testis in the most studied species is given in table 2. A comparison of the data in table 3 favours the conclusion that testosterone secretion, at its onset, is independent of LH. Later on, it comes under LH control, as shown in the rat foetus by decapitation experiments. It should be added that testosterone

TABLE 3

Time of onset of testosterone and LH secretion in foetal mammals.

Species	Testosterone secretion	LH secretion
Mouse	12 days	14 to 16 days
Rat	15 days	17 days
Rabbit	17 to 19 days	19 days
Human	8 to 10 weeks	10 to 12 weeks

secretion in the human foetal testis may be initiated by hCG (Winter *et al.*, 1981), although the presence of LH/hCG receptors before week 8 of gestation has to be demonstrated.

d. *Control of testosterone secretion by LH.* — The testes from 20-day old rat foetuses decapitated 1 day earlier produced 5-fold less testosterone *in vitro* than control testes. When decapitation was performed on day 20, testosterone production measured on day 21 had decreased 8-fold (Weniger *et al.,* 1977 ; Naessany *et al.,* 1981). LH given to the foetus at the time of decapitation restored

normal testosterone production (Chouraqui *et al.,* 1977). Decapitation on day 18 did not alter testosterone production on day 19 (Habert and Picon, 1982). So, testosterone secretion by foetal rat testes is regulated by LH from 19 days on.

e. *Hypothalamo-pituitary-testicular interrelationships.* — The next studies tried to determine the existence of an inhibitory action of testosterone on LH secretion. After 3 injections of dihydrotestosterone to pregnant rabbits at 12-hour intervals, the testicular testosterone content measured 5 h after the last injection had not varied in comparison with control values when the foetuses were sacrificed at 23 days, but it had decreased by one-half at 25 days. So, a negative feedback mechanism of testosterone on LH secretion is established in rabbit foetuses between days 23 and 25 (Veyssière *et al.,* 1980a).

A similar reduction in the testosterone content of foetal testes was observed after testosterone injection to pregnant rats (Naessany *et al.,* 1981) ; this negative feedback becomes effective on day 19 (Naessany and Picon, 1982).

That a drop in serum testosterone results in an increase in LH secretion has been demonstrated in rhesus monkey foetuses. Three weeks after orchiectomy on day 100, the serum LH concentration rose 10-fold (Ellinwood *et al.,* 1982). So, the existence of testicular-pituitary interrelationships is a well-established feature in mammalian foetuses.

These relationships probably extend to the hypothalamus, as is suggested by the following results. In 6-month old bovine foetuses, a 2-fold increase in serum testosterone concentration was observed 3 h after GnRH injection (Kiser *et al.,* 1975). In 4 to 5-month old rhesus monkey foetuses, mean serum testosterone concentration had increased 2 to 2.5-fold by 1 hour after GnRH administration (Huhtaniemi *et al.,* 1977b ; Norman and Spies, 1979). In 29-day old rabbit foetuses, the plasma testosterone level had risen 8-fold and the testicular testosterone content 3.5-fold by 30 min after GnRH injection (Veyssière *et al.,* 1977).

2. Oestrogen production.

a. *By foetal testis.* — The aromatization of ^{3}H-labelled androgens by the 19 to 20-day old foetal rat testis has been reported by Weniger and Zeis (1983, 1985). The regulation of this process by gonadotrophins is under current investigation.

b. *By immature testis.* — Results of experiments with testes from immature rats are interesting (Tsai-Morris *et al.,* 1985). In 5-day old neonatal rats, aromatase activity, measured by the ^{3}H H_2O release method, is as high in isolated Leydig cells as in isolated Sertoli cells. At the age of 15 days, it is twice as high in Leydig cells as in Sertoli cells. At 60 days, the ratio is 5 : 1. So, aromatase activity increases with age in Leydig cells and decreases in Sertoli cells.

These results of Tsai-Morris *et al.* (1985) confirm results obtained previously in limited studies. For instance, FSH, (but not LH) increased cAMP levels in

Sertoli cell preparations from 18 to 20-day old rats (Dorrington et al., 1975a). FSH and cAMP (but not LH) greatly stimulated the conversion of exogenous testosterone into oestradiol in Sertoli cell aggregates from 10 to 20-day old rats cultured for 4 to 48 h (Dorrington and Armstrong, 1975a, b, 1979 ; Verhoeven et al., 1979 ; Gore-Langton et al., 1980 ; Suárez-Quian et al., 1983). So, it is established that Sertoli cells from 5 to 20-day old rats possess aromatase activity, which can be stimulated by FSH. The production of oestradiol by the Leydig cells has been less thoroughly investigated. However, the results of Tsai-Morris et al. (1985) clearly show that aromatase activity exists in Leydig cells and can be stimulated by hCG.

B. The ovary

1. Androgen production.

a. By foetal ovary. — LH or hCG markedly stimulated the secretion of androgens by 32 to 37-day old foetal pig ovaries in vitro. These androgens were formed from endogenous or exogenous precursors. Androstenedione was formed almost exclusively, testosterone amounting to less than 5 % (Raeside, 1983).

b. By immature ovary. — Studies in immature animals confirm the stimulatory effect of LH on androgen secretion. Seven-day old mouse ovaries formed mostly androstenedione (Fortune and Eppig, 1979), while 25-day old hamster ovaries formed approximately equal amounts of testosterone and androstenedione (Shaha and Greenwald, 1983).

In immature hamster ovaries, the conversion of androstenedione into testosterone was enhanced under the influence of FSH which stimulated 17 β-hydroxysteroid-dehydrogenase activity. 5 α-Reductase activity was enhanced by LH (Tsuji et al., 1983).

2. Oestrogen production.

a. By immature ovary. — It is preferable to begin by analysing experiments on prepubertal ovaries.

The fundamental work is that of Moon et al. (1975). These authors showed that ovaries from 28-day old hypophysectomized rats cultured in Medium 199 secreted oestradiol into the culture medium. The addition of testosterone was virtually without effect. On the contrary, FSH produced a 2 to 3-fold increase in œstradiol secretion. In the presence of 0.5 μM testosterone as a substrate, FSH had a still greater effect and enhanced oestradiol production 8 to 10-fold. It was concluded that FSH stimulated aromatization.

In subsequent experiments, the same authors (Dorrington *et al.,* 1975b) showed that granulosa cells isolated from the ovaries of hypophysectomized 29-day old rats secreted only negligible quantities of oestradiol in the absence of both testosterone and FSH. The addition of testosterone or FSH separately to the culture medium did not influence oestradiol production. However, the addition of both substances greatly enhanced oestradiol secretion. Oestrone was produced too, but at levels only 10 to 30 % as high as oestradiol. Taking into acount these results and those of the previous work, it was concluded that granulosa cells needed both androgen precursors provided by theca and/or interstitial cells and FSH which specifically stimulates the aromatizing enzyme system.

The results of Dorrington *et al.* (1975b) were confirmed by Erickson and Hsueh (1978). In the absence of androstenedione as a substrate, no significant stimulation of oestrogen secretion was observed in FSH-treated granulosa cell cultures. However, in the presence of androstenedione, FSH stimulated oestrogen production 9 and 40-fold after 1 and 2 days, respectively.

Funkenstein and Nimrod (1983) studied the steroidogenic capabilities of 4-day old rat ovaries in organ culture. In the presence of 3 μM testosterone, there was significant œstradiol production which increased 4-fold when $(Bu)_2cAMP$ was added to the medium. Oestrone was not measured.

In neonatal mouse ovaries, oestradiol secretion was stimulated by FSH at the 7-day stage but not at the 2-day stage (Fortune and Eppig, 1979).

b. *By foetal ovary.* — The first of a series of studies on oestrogen formation by the foetal ovary of mammals was that of Milewich *et al.* (1977) in rabbits. These authors determined the conversion percentages of 0.5 μM ^{3}H-testosterone or ^{3}H-androstenedione to oestrone and oestradiol by the 19-day old ovary *in vitro.* The ratios of oestradiol to oestrone formed from testosterone and androstenedione, respectively, were 15 and 7.5. Using the same methods, George and Wilson (1978) found that the ovary of the 12.5 to 13.8-cm CR length human foetus also formed more oestradiol than oestrone ; the ratio was about 2 with androstenedione as the substrate and 8 with testosterone.

According to Terada *et al.* (1984), aromatization of ^{3}H-androstenedione could not be effected by the 18-day old mouse ovary in organ culture. However, in the presence of FSH, the production of oestrone and oestradiol was already detected at the 17-day stage, when equal amounts of both oestrogens were formed.

When oestrogen production by the 19 to 20-day old foetal rat ovary was investigated, it was found that appreciable amounts of oestrone were formed from either testosterone or progesterone, even in the absence of FSH ; virtually no oestradiol was formed (Weniger *et al.,* 1984). The effect of FSH was studied in the 19-day old ovary ; a stimulatory effect on oestrogen formation with either progesterone, dehydroepiandrosterone, testosterone or androstenedione could not be demonstrated (Weniger *et al.,* 1985). However, Picon *et al.* (1985) found that FSH enhanced the aromatase activity of the 20-day old ovary.

For the time being, the case of the foetal rat ovary appears to be unique as regards the prevalence of the formation of oestrone over that of oestradiol. Since, according to Dorrington *et al.* (1975b), the production of oestradiol is 3 to

10-fold as high as that of oestrone at the 29-day stage after birth, a shift must occur between that stage and the late foetal stages. It will be interesting to determine a more exact timing for this shift. On the other hand, the stimulatory effect of FSH on aromatase activity in the foetal mouse and rat ovary needs to be confirmed.

Conclusion.

Gonado-pituitary interrelationships already exist during foetal life in mammals. At its onset, androgen secretion by the foetal testis is independent of pituitary LH but, later, it comes under LH control. Testosterone exerts a retroinhibition on LH secretion. On the other hand, oestrogen secretion by the foetal testis has been demonstrated.

The ovary secretes androgens as well as oestrogens. An interesting feature of the rat ovary is the shift from oestrone to oestradiol secretion between foetal and prepubertal life. The stimulatory effect of FSH on the aromatizing enzyme system in the ovary has been shown in some studies but not in others.

Résumé. *Sécrétion de stéroïdes par les gonades fœtales de mammifères.*

Les principales conclusions qui se dégagent de la présente mise au point sont les suivantes.

Des corrélations hypophyso-gonadiques existent dès la vie fœtale chez les mammifères. A l'origine, la sécrétion de testostérone par le testicule fœtal est indépendante d'une stimulation hypophysaire, mais elle tombe sous la dépendance de LH avant la fin de la vie fœtale. La testostérone exerce une rétroaction inhibitrice sur la sécrétion de LH. D'autre part, on a démontré la sécrétion d'œstradiol par le testicule fœtal.

L'ovaire sécrète à la fois des androgènes et des œstrogènes. Chez le rat, il est intéressant de noter le passage d'une prédominance de sécrétion d'œstrone à une prévalence de sécrétion d'œstradiol entre le stade fœtal et le stade de la maturité sexuelle. L'action stimulatrice de FSH sur l'aromatase de l'ovaire fœtal est controversée.

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