Effect of follicular steroids on the maturation and fertilization of mammalian oocytes

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SUMMARY

Pronuclear development was used to measure the effects on ovine oocytes of altering follicular steroidogenesis during maturation in vitro. Follicular steroid secretion was altered using enzyme inhibitors and exogenous steroid supplementation. Abnormalities induced during maturation were measured 24 h after transfer of oocytes to the oviducts of inseminated hosts.

The presence throughout maturation of aminoglutethimide, an inhibitor of the conversion of cholesterol to pregnenolone, reduced steroid secretion to 7% of that in controls and decreased from 77% to 33% the number of normal oocytes. Abnormalities were substantially reduced by the addition of aminoglutethimide during the final 8 h of maturation only.

The inhibition of 17α-hydroxylase enzymes with SU10603 reduced oestrogen and testosterone secretion to about 10% of control levels but had no effect on progesterin secretion. Only 13% of oocytes matured in the continual presence of SU10603 underwent normal fertilization. The number of oocytes undergoing normal fertilization was increased to about 50% by (i) delaying the addition of SU10603 until the last 8 h of the maturation period or (ii) adding exogenous steroids to follicles cultured with inhibitor from explantation.

It is concluded that oocytes require a specific intra-follicular steroid environment for the completion of the full maturation process. Alterations to the steroid profile during maturation induce changes in the oocyte which are expressed as gross abnormalities at fertilization.

INTRODUCTION

The importance of steroids in the maturation of mammalian oocytes is controversial. It is the opinion of some workers that steroids are not involved in maturation since steroid inhibitors do not prevent the resumption of meiosis in follicles treated with LH in vitro (Lieberman et al. 1976; Tsafriri, Lieberman, Ahren & Lindner, 1976). Other workers have, however, reported that steroids are important in mammals for the completion of maturation changes in both the nucleus and cytoplasm of the oocyte (Bae & Foote, 1975; McGaughey, 1977; Thibault, 1977; Moor & Warnes, 1978). The central role played by progesterone and its metabolites in the regulation of oocyte maturation in other species such as amphibia and fish is already firmly established (Masui, 1967; Smith, Ecker & Subtelny, 1968; Jalabert, 1976).

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This paper provides new evidence for the involvement of steroids in the full maturation of mammalian oocytes. Our approach has been to determine the developmental consequences of selectively altering follicular steroidogenesis during oocyte maturation. The necessary regulation of follicular steroidogenesis has been obtained by the direct application of steroid inhibitors and steroid supplementation to isolated follicles in vitro. Physiological relevance has been ensured by using methods of follicle culture which lead to full maturation of the oocytes as judged by their subsequent development into viable young (Moor & Trounson, 1977). Manipulation of the steroid environment induces subtle intracellular changes in oocytes, which although not detectable by biochemical analysis, were nevertheless expressed in a measurable form at fertilization.

**MATERIALS AND METHODS**

**Preparation and culture of follicles**

Over 500 non-atretic follicles (3.5-5.5 mm in diameter) were dissected from the ovaries of Welsh Mountain sheep which had been injected with 1200 i.u. pregnant mare serum gonadotrophin (PMSG) on day 8-10 of the cycle and slaughtered 36-40 h later. Follicular fluid was obtained from 19 follicles immediately after dissection. The other intact follicles were placed singly on stainless-steel grids in plastic culture dishes and cultured under hyperbaric conditions using the conditions and culture medium described by Moor & Trounson (1977). Cultured follicles were divided into groups and exposed to one of eight different treatments, outlined in Table 1, which varied with respect to the types of gonadotrophins, steroids and steroid enzyme inhibitors added to the basic culture medium. Gonadotrophins were added in the following standard combination; follicle-stimulating hormone (NIH-FSH-S12, 5 μg ml⁻¹), luteinizing hormone (NIH-LH-S18, 3 μg ml⁻¹) and prolactin (NIH-P-S9, 0.02 μg ml⁻¹). Steroid supplementation consisted of either oestradiol, 17β (1 μg ml⁻¹) alone or in combination with the following steroids, oestradiol-17β (μg ml⁻¹), testosterone (1 μg ml⁻¹), androstenedione (1 μg ml⁻¹), 17β-hydroxyprogesterone (1 μg ml⁻¹) and progesterone (1 μg ml⁻¹).Steroid synthesis in follicles was blocked at one of two sites in the biosynthetic pathway: (i) by inhibiting the conversion of cholesterol to pregnenolone using the 20α-cholesterol oxidase inhibitor, aminoglutethimide at a concentration of 10⁻³ M (Kahnt & Neher, 1966) or (ii) by inhibiting the 17α-hydroxylase enzyme system with 7-chloro-3,4-dihydro-2(3-pyridyl)-1-(2H)-napthalenone (SU-10603) at a concentration of 10⁻⁴ M (Chart, Sheppard, Mowles & Howie, 1962).

Inhibitors which were used in preliminary tests but not included in the main study because of cell toxicity, incomplete enzyme inhibition or slow action included the 7-dehydrocholesterol reductase inhibitor AY9944 (Ayerst Laboratories), and the 3β-hydroxysteroid dehydrogenase inhibitors Trilostane (Sterling-Winthrop Research Institute) and Oxymethalone (Syntex Laboratories).
Table 1. *The number of cultured oocytes transferred to the oviducts of inseminated recipients and the number and appearance of the oocytes 24 h after transplantation*

(The following supplements were added in different combinations or at different stages during maturation to follicles in the eight different experiments: (a) gonadotrophins (FSH, 5 μg; LH, 3 μg; prolactin, 0.02 μg ml⁻¹ medium), (b) steroids all added at 1 μg ml⁻¹ (oestradiol, 17β, E₂ 17β; testosterone, T; androstenedione, Ad; 17α-hydroxyprogesterone, 17αHP; progesterone, P), and steroid enzyme inhibitors (aminogluthethimide, 10⁻³ M; SU10603, 10⁻⁴ M).)
Transplantation and evaluation of cultured oocytes

After 24 h in culture, oocytes together with their associated cumulus cells were removed from the follicles and placed in dissection medium at 37 °C (Dulbecco's phosphate-buffered saline supplemented with bovine serum albumin, 4 mg ml⁻¹; pyruvate, 0.36 mM; lactate 23.8 mM and glucose 5.5 mM). Transfer of oocytes to the oviducts of sheep in oestrus was completed within 30–45 min of dissection (Moor & Trounson, 1977). The randomized design of the experiments resulted in oocytes from two different treatments being transferred to almost every host animal. Freshly ejaculated semen showing wave-like motility was introduced into each uterine horn (0.01–0.02 ml containing 2–4 × 10⁷ spermatozoa) of recipients immediately before transfer of oocytes. Eggs were recovered from the recipients 24 h after transplantation. Eggs were prepared as whole mounts and examined under phase-contrast microscopy for sperm attachment before being fixed in acetic acid : ethanol (1:3, v/v), stained with lacmoid and subjected to more detailed microscopical examination.

Measurement of steroids in the follicular fluid

Steroids were extracted from the follicular fluid with redistilled diethyl ether (100:1, v/v). The progesterone and total unconjugated oestrogen content of the ether extracts were measured by radioimmunoassay using methods and antisera described previously (Moor, Hay, McIntosh & Caldwell, 1973). The cross-reactivity of the progesterone antiserum estimated at 50% displacement of labelled progesterone, was, with the exception of pregnenolone (3-5%), less than 1% for all the major steroids secreted by the ovine follicle (Seamark, Moor & McIntosh, 1974). The cross-reactivity of the oestradiol-17β antiserum was less than 1% for the C₁₉ and C₂₁ steroids; cross-reactivity with the C₁₈ steroids was oestrone, 55%; oestradiol-17α, 6% and oestrone sulphate, 4%. Validation of the radioimmunoassay used for testosterone determination is contained in the paper by Moor (1977). The cross-reactivity of the testosterone antiserum with C₁₈ and C₂₁ steroids was less than 0.01%, but for the C₁₉ steroids was 5α-dihydrotestosterone, 100%; 5β-androstane-3α,17β-diol, 43%; 5α-androstane-3α,17β-diol, 41%; androst-5-en-3β,17β-diol, 4% and androstenedione, 0.01%. The term ‘testosterone’ is used in this paper because previous studies have shown that the androgens which cross-react with our testosterone antibody account for less than 5% of the steroid in that fraction. The minimum amount of steroid that could be distinguished from the blank was 10 pg for oestrogen, 15 pg for testosterone and 30 pg for progesterone. The within-assay and between-assay coefficients of variation for the three assays were 6.1% and 12% for progesterone, 5.9% and 7% for oestrogen and 4.7% and 8% for testosterone.
Steroid–oocyte interactions

RESULTS

Sheep oocytes, removed from follicles after a 24 h culture period in defined conditions, become fertilized and develop into young when transferred to inseminated recipients (Moor & Trounson, 1977). The groups of follicles outlined in Table 1 were cultured under the same conditions as the above except that follicular steroidogenesis was selectively altered by enzyme inhibitors and steroid supplementation. Oocytes from 501 follicles cultured in the eight groups were transferred to inseminated recipients and 24 h later 458 (91 %) oocytes were recovered from the oviducts of the recipients. On examination, 12 % of the recovered oocytes were classified as degenerate and 11 % as aspermic. Both of these types of oocytes were excluded from further consideration and all subsequent analyses were restricted to the 355 (77 %) oocytes that had at least one spermatozoon attached to the zona pellucida or embedded in the cytoplasm.

Oocyte development after inhibition of total steroid synthesis

It is clear from Table 2 that aminoglutethimide, added to the culture medium at explantation sharply reduced \( P < 0.001 \) the secretion of all the major classes of steroids secreted by follicles. By contrast, addition of aminoglutethimide 16 h after explantation markedly depressed progesterone and testosterone secretion below that of controls \( P < 0.001 \) but had little effect on the levels of oestrogen. This latter finding is readily explained by the fact that oestrogen secretion occurs in the early phase of maturation process while progestin secretion is confined to the last phase.

Figure 1 summarizes the response of the oocyte, as measured by changes during fertilization, of totally inhibiting steroid secretion during maturation in vitro. Oocytes from follicles cultured in the absence of inhibitors (control group, Fig. 1A) showed normal development of male and female pronuclei in the great majority of oocytes (77 %). In these normal oocytes, both the male and female pronuclei had generally developed to the late pronuclear stage and the mid-piece of the sperm tail was clearly visible (Fig. 2). A few oocytes had reached the stage of syngamy (Figs 3, 4). The incidence of polyspermy in oocytes with normal development of the female pronucleus was negligible. Thirteen oocytes (19 %) failed to complete meiosis. Nine had not developed beyond metaphase-I and four, which had been penetrated by spermatozoa, remained at metaphase-II.

Inhibiting steroidogenesis by adding aminoglutethimide at explantation reduced to only 33 % the proportion of oocytes with morphologically normal pronuclear development (Fig. 1B). The most striking abnormality in this group was the large proportion of oocytes that failed to complete meiosis. The number of oocytes blocked at or before metaphase I amounted to 33 %, but in a further 22 % of oocytes the chromosomes remained at metaphase II.
Table 2. Mean concentration (± S.E.M.) of unconjugated oestrogen, testosterone and progesterone in follicular fluid at explantation and after 24 h culture in medium supplemented with gonadotrophins and inhibitors of steroid synthesis (see Table 1 for details)

<table>
<thead>
<tr>
<th>Period of culture (h)</th>
<th>Enzyme inhibitor</th>
<th>Period of inhibition (h)</th>
<th>No follicles</th>
<th>Follicular fluid steroid concentration (pmoles ml⁻¹)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Inhibitor</td>
<td></td>
<td></td>
<td>Oestrogen</td>
</tr>
<tr>
<td>0</td>
<td>—</td>
<td>—</td>
<td>29</td>
<td>2485 ± 186</td>
</tr>
<tr>
<td>24</td>
<td>—</td>
<td>—</td>
<td>74</td>
<td>3729 ± 517</td>
</tr>
<tr>
<td>24</td>
<td>Aminoglutethimide (10⁻³ M)</td>
<td>0–24</td>
<td>71</td>
<td>411 ± 66</td>
</tr>
<tr>
<td>24</td>
<td>Aminoglutethimide (10⁻³ M)</td>
<td>16–24</td>
<td>47</td>
<td>2686 ± 161</td>
</tr>
<tr>
<td>24</td>
<td>SU10603 (10⁻⁴ M)</td>
<td>0–24</td>
<td>66</td>
<td>525 ± 128</td>
</tr>
<tr>
<td>24</td>
<td>SU10603 (10⁻⁴ M)</td>
<td>16–24</td>
<td>23</td>
<td>7461 ± 895</td>
</tr>
</tbody>
</table>
Steroid–oocyte interactions

Fig. 1. Effect of blocking steroid synthesis during maturation in vitro on the development of oocytes examined 24 h after transfer to inseminated hosts. Illustrated are the percentage of oocytes at the germinal vesicle (GV), first and second metaphase (MI, MII) or pronuclear (PN) stages of development, together with the associated extent of sperm development in oocytes at each stage of meiosis. Sperm development is classified as: attachment but no penetration (■), monospermic penetration with abnormal decondensation (□), polyspermic penetration (□□) and normal male pronuclear formation (●).

The following supplements were added to the follicles during the 24 h maturation period. (+) Gonadotrophin added at explantation (control group); (B) gonadotrophin and aminoglutethimide ($10^{-3}$ M) added at explantation; (C) gonadotrophin at explantation and aminoglutethimide ($10^{-3}$ M) added 16 h later; (D) gonadotrophin, aminoglutethimide ($10^{-3}$ M) and exogenous steroids added at explantation.
following sperm penetration (Fig. 5). The majority of these oocytes were penetrated by more than one spermatozoon and the development of the male pronuclei was also abnormal. In some oocytes the male pronuclei were arrested at the stage of sperm head swelling and decondensation (Fig. 6) and in others the pronuclei appeared to be misshapen and fragmenting (Fig. 7).

Addition of aminogluthethimide 16 h after explantation (Fig. 1C) increased the number of oocytes with normal pronuclei from 33 % (when aminogluthethimide was added at explantation) to 59 %. Female pronuclear development occurred normally in 88 % of all oocytes. None was blocked at metaphase II after sperm penetration, but in 12 % meiosis had not progressed beyond metaphase I.

A similar high incidence (76 %) of normal female pronuclear development occurred in oocytes from follicles cultured from explantation with aminogluthethimide but with exogenous steroid supplementation (Fig. 1D). A further 14 % of oocytes were at metaphase II but these had not been penetrated by spermatozoa. There was a relatively high incidence of polyspermy (22 %) and this together with another 11 % of the oocytes in which male pronuclear formation was retarded (Fig. 9) resulted in only 41 % containing normal male and female pronuclei.

**Oocyte development after inhibition of 17a-hydroxylase**

As expected, inhibition of the 17α-hydroxylase system with SU10603 sharply reduced oestrogen and androgen secretion (∼P < 0.001) but had no apparent effect on the output of progesterone (see Table 2). With the exception of oestrogen which was increased above control levels (∼P < 0.01), other steroid

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**Figures 2–7**

Figs. 2–4. Normal sequence of nuclear events during fertilization of oocytes matured in control follicles in vitro (see Fig. 1A). Scale bar represents 20 μm.

Fig. 2. Male and female pronucleus together with the midpiece from the fertilizing spermatozoon.

Fig. 3. Oocyte at syngamy.

Fig. 4. Oocyte showing first cleavage division.

Figs. 5–7. Abnormalities during fertilization induced in oocytes by the inhibition of steroid synthesis during maturation with aminogluthethimide (see Fig. 1B). Scale bar represents 20 μm.

Fig. 5. Oocyte nucleus blocked at metaphase II and showing the first polar body. An abnormal male pronucleus in this polyspermic oocyte is shown in Fig. 7.

Fig. 6. Polyspermy and arrested sperm decondensation. Development of the female nucleus in this oocyte was arrested at metaphase II.

Fig. 7. An abnormal and fragmenting male pronuclei in the oocyte depicted in Fig. 5. A second penetrated sperm shows almost no decondensation or swelling of the sperm head.
secretion was unaffected by the addition of SU10603 at 16 h after explantation of follicles.

The addition of the 17α-hydroxylase inhibitor, SU10603, at explantation resulted in an almost total disruption of normal oocyte development and fertilization (Fig. 8A). The abnormalities were characterized by (i) an inability of spermatozoa to penetrate the zona pellucida after attachment to it (73% not penetrated) and (ii) by a block in meiosis in 57% of oocytes at or before the formation of the first metaphase plate (Figs 10, 11).

The deleterious effect of SU10603 was considerably reduced both by delaying the addition of the inhibitor for 16 h (Fig. 8B) and by supplementing the medium containing SU10603 with exogenous steroids (Fig. 8C). Oocytes from follicles exposed to SU10603 between 16 and 24 h after explantation had mostly developed to metaphase II or had formed pronuclei (93%). Only 20% had not been penetrated by spermatozoa and the majority of the pronuclear eggs were normal. The presence throughout culture of exogenous steroids together with SU10603 also decreased problems of sperm penetration (only 12% not penetrated) and increased the proportion of oocytes in which meiosis had been completed (71% at metaphase II or pronuclear stage). Normal male and female pronuclei were present in 51% of oocytes, but the majority of the immature oocytes that had been penetrated by spermatozoa were polyspermic (Fig. 12).

The formation of normal male and female pronuclei occurred in only 5% of oocytes obtained from follicles cultured in the absence of gonadotrophins but in the presence of exogenous steroids (Fig. 8D). Meiosis was blocked at or before metaphase I in 18% of oocytes and although 52% had reached metaphase II at the time when they were recovered from the recipient animals, a large proportion of these had not been penetrated by spermatozoa. A relatively high proportion of the pronuclear eggs were also polyspermic and many of the male pronuclei in these oocytes as well as in the immature oocytes penetrated by spermatozoa showed irregularities in decondensation and were fragmenting (Figs 13, 14).

**DISCUSSION**

The results demonstrated that alterations to the normal profile of steroids secreted during maturation induced intracellular changes in oocytes which are expressed clearly at fertilization. The different abnormalities that develop during the period of interaction between the sperm and oocyte reflect the type of steroid environment pertaining during maturation. It would seem that imbalances in the steroid profile affect oocyte maturation more severely than the total inhibition of steroid biosynthesis.

The addition of aminogluthethimide effectively reduced overall follicular steroid secretion and sharply increased the incidence of abnormalities within the oocyte. These results cannot, however, for the following reasons be inter-
Fig. 8. Effect of selectively altering the profile of steroids during maturation in vitro on the development of oocytes examined 24 h after transfer to inseminated hosts. Illustrated are the percentages of oocytes at the germinal vesicle (GV), first and second metaphase (M I, M II) and pronuclear stages of development, together with the associated extent of sperm development in oocytes at each stage of meiosis. Sperm development is classified as: attachment but not penetration (□); monospermic penetration with abnormal decondensation (■); polyspermic penetration (□); and normal male pronuclear formation (■).

The following supplements were added to the follicles during the 24 h maturation period: (A) gonadotrophin and SU10603 (10^{-4} M) added at explantation; (B) gonadotrophin at explantation and SU10603 (10^{-4} M) 16 h later; (C) gonadotrophin, SU10603 (10^{-4} M) and exogenous steroid added at explantation; (D) exogenous steroid added at explantation.
interpreted as measuring the capacity of oocytes to mature in the total absence of steroids. Firstly, it has been found that the block to follicular steroidogenesis induced by aminoglutethimide requires a few hours to become effective (H. M. Dott, D. Green & R. M. Moor, unpublished observations). Secondly, the high levels of secreted steroid contained within the follicle (see Table 2) are not altered or removed by the addition of the inhibitor. Finally, it is evident, from the present results that aminoglutethimide is effective in disrupting oocyte development during the first phase of the maturation process only; addition of the inhibitor during the second phase of maturation was without effect on subsequent pronuclear development. It is therefore probable that the period during which the oocyte is most sensitive to steroid deprivation coincides in aminoglutethimide treated follicles with the period of highest residual steroid availability. Thus, the real effect on the oocyte of a total absence of steroids may be considerably more severe than is suggested by the results of our experiments. This possibility is supported by preliminary evidence which suggests that the addition of antisera to oestrogen and testosterone, together with aminoglutethimide, induces more abnormalities in oocytes than the inhibitor alone (R. M. Moor, unpublished observations).

A comparison of the effects of blocking either the 20α cholesterol or the 17α-hydroxylase system indicates that the maturing oocyte is probably more sensitive to an imbalance rather than an overall reduction in steroid levels. This postulate accords with the report by Soupart (1974) that human oocytes matured \textit{in vitro} require a sequence of steroid additions in order to develop fully the potential to sustain subsequent normal male pronuclear development.

\textbf{Figures 9–14}

Abnormalities during fertilization induced in oocytes by selectively altering steroid concentrations in follicles during maturation. Scale bar represents 20 μM.

Fig. 9. Female pronucleus and penetrated spermatozoon with arrested development in an oocyte cultured with aminoglutethimide and exogenous steroid supplementation (see Fig. 1D).

Fig. 10. Oocyte nucleus at the germinal vesicle stage and numerous spermatozoa which had become embedded in the zona pellucida (removed during fixation) but had failed to enter the cytoplasm of an oocyte matured in the presence of SU10603 (see Fig. 8A).

Fig. 11. Oocyte nucleus arrested at metaphase I and spermatozoa attached to the zona pellucida (removed during fixation); spermatozoa were unable to penetrate oocytes matured in the presence of SU10602 (see Fig. 8A).

Fig. 12. Oocyte nucleus at the germinal vesicle stage with polyspermy and sperm nuclei showing arrested swelling and decondensation in an oocyte matured with SU10603 and exogenous steroids (see Fig. 8C).

Fig. 13. Fragmentation of the male pronucleus in a oocyte cultured with exogenous steroids but without gonadotrophic support (see Fig. 8D).

Fig. 14. Metaphase II chromosomes and first polar body together with an abnormal pronucleus in an oocyte matured with exogenous steroids but without gonadotrophic support (see Fig. 8D).
The requirement for a strictly regulated sequence of steroid changes during maturation may explain why exogenous steroids did not completely reverse the action of the inhibitors. The addition of steroids together with amino-glutethimide restored female pronuclear development to control levels but did not restore normal male pronuclear development. This incomplete reversal could have been caused by the addition at explantation of equal amounts of oestrogen, androgens and progestins; the resultant steroid environment clearly differed substantially from that pertaining in vivo (Moor, 1978). By contrast only oestrogen, androgen and 17α-hydroxylated progestins were included when the 17α-hydroxylase inhibitor was used; the resultant more normal steroid profile may explain the higher incidence of reversal in that experiment. Steroids other than those supplied (the follicle secretes at least 12–16 different steroids) or the precise concentration of added steroid may also be of significance in oocyte maturation. With the numerous steroid variables likely to be important in oocyte maturation it is perhaps unrealistic to expect complete reversal of enzyme inhibition until considerably more detailed information on steroid-oocyte interactions becomes available.

It is possible that some of the abnormalities observed in oocytes exposed to the inhibitors used in this study could have resulted from direct toxic effects of the drugs. While such direct cytotoxic effects cannot be discounted, the following observations provide evidence against this possibility. Firstly, oocytes exposed to both inhibitors develop entirely normally provided only that the drugs are not added until the second phase of maturation. Secondly, the simple addition of steroids to cultures containing one of the inhibitors (SU10603) increases from 13 to 50% the number of oocytes undergoing normal development and fertilization. Thirdly, morphological studies of the cumulus, granulosa and theca of follicles treated with these inhibitors provided no evidence of cell damage (M. F. Hay & R. M. Moor, unpublished observations).

The precise mechanism by which steroid imbalances affect the oocyte is unclear. It seems unlikely that maturation in mammalian oocytes is initiated by the same kind of steroid–membrane interactions that initiate maturation in amphibian oocytes (Godeau, Schorderet-Slatkine, Hubert & Baulieu, 1978). A favoured alternative hypothesis for mammals suggests that the disruption of gap junctions between cumulus cells and oocytes initiates maturation by reducing intercellular uptake into oocytes of inhibitors which block meiosis; the most likely inhibitor being cyclic AMP (Anderson & Albertini, 1976; Gilula, Epstein & Beers, 1978; Dekel & Beers, 1978). Recent experiments on the role of hormones in gap junction function suggest that steroids have a minor but statistically significant effect on the passage of molecules from cumulus cells to oocytes (Moor, Smith & Dawson, 1979). However, the major regulators of intercellular coupling and amino acid transport across the oolemma, namely FSH and LH, are not dependent upon steroid synthesis for their action on the membrane (Moor & Smith, 1978, 1979; Moor et al. 1979). It therefore seems
unlikely that the action of steroids is primarily on the membrane of the oocyte.

The early sequence of maturational changes in the nucleus leading to germinal vesicle breakdown and formation of the first metaphase plate are allegedly independent of steroid support (Lieberman et al. 1976). The evidence of McGaughey (1977) indicates, however, that the absence of steroids during the later stages of maturation increases the incidence of chromosomal abnormality especially at telophase I and metaphase II. The present results support the observations of McGaughey (1977) and show that major changes in follicular steroidogenesis during maturation result in severe nuclear aberrations. The addition of exogenous steroids together with the steroid inhibitor, particularly when aminogluthethimide is used, reduce substantially the incidence of chromosomal abnormalities in the oocytes.

Oocytes, exposed before maturation to sperm, become penetrated but lack the cytoplasmic factors that induce decondensation and formation of the male pronucleus (reviewed by Thibault, 1977). A specific factor responsible for the transformation of the sperm into a male pronucleus has not been characterized but is probably amongst those compounds whose synthesis is induced during normal oocyte maturation (Golbus & Stein, 1976; Schultz & Wasserman, 1977; Warnes, Moor & Johnson, 1977; Van Blerkom & McGaughey, 1978). Evidence from Soupart (1974) and Thibault, Gerard & Menezo (1975) suggests that steroids are important for the synthesis of the so called pronucleus growth factor (MPGF). Our results support this contention.

We have used developmental criteria in this study to detect abnormalities during oocyte maturation because of the extreme sensitivity of such biological tests. It has been our purpose to extend the study by correlating developmental aberrations with specific synthetic changes in the oocyte. We have, however, so far failed to detect clear steroid-dependent changes in protein synthesis in oocytes using polyacrylamide slab gels containing sodium dodecyl sulphate (R. M. Moor & G. M. Warnes, unpublished observations). The substantially greater degree of protein separation obtained using isoelectric focusing and SDS polyacrylamide electrophoresis (O’Farrell, 1975), may however reveal differences in synthesis which were not resolved by one-dimensional gel electrophoresis.

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