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1982
EXCERPTA MEDICA, AMSTERDAM-OXFORD-PRINCETON

International Congress Series No. 560
ISBN Excerpta Medica 90 219 0505 1
ISBN Elsevier North-Holland 0 444 90231 7

Library of Congress Cataloging in Publication Data
Main entry under title:

Follicular maturation and ovulation.

(International congress series ; no. 560)
Includes indexes.
1. Graafian follicle--Congresses. 2. Ovulation--Congresses. I. Rolland, R. II. Title. III. Series. [DNLM: 1. Graafian follicle--Growth and development--Congresses. 2. Ovulation--Drug effects--Congresses. 3. Oogenesis--Drug effects--Congresses. W3 RE4424 4th 1981f / WP320 R372 1981f]
QP261.F64 599.01'6 82-1364
ISBN 0-444-90231-7 (Elsevier North-Holland)
AACR2

Publisher:
Excerpta Medica
P.O. Box 211
1000 AE Amsterdam
P.O. Box 1126

Sole Distributors for the USA and Canada:
Elsevier North-Holland Inc.
52 Vanderbilt Avenue
New York, N.Y. 10017

Printed in The Netherlands by Casparie - Amsterdam

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IVth REINIER DE GRAAF SYMPOSIUM 'FOLLICULAR MATURATION AND OVULATION'

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Preface

Our knowledge in the field of reproductive biology has expanded tremendously during the last few decades. On the one hand this is due to the development of scientific research in general and in the field of reproduction in particular, but on the other hand much of the gathered knowledge comes from clinical studies. This rapid expansion of knowledge has the risk of broadening the gap between scientists involved in basic research and the more clinically oriented research workers. Furthermore, young investigators are faced with an enormous output of recent papers and this may lead to limitation of their knowledge of research carried out in the past.

Although the first Reinier de Graaf Symposium in Noordwijkerhout in 1973 was held to commemorate the tercentenary of Reinier de Graaf's death, the underlying hope of the organizers was that of bringing together researchers from basic science and clinicians to exchange and expand their knowledge on the function of the ovary. The first symposium was a great success and it stimulated the organizers to carry on with their work and since then Reinier de Graaf Symposia were held in Amsterdam (1975), Maastricht (1978) and finally in Nijmegen (1981). At each symposium a certain topic on ovarian function is selected as main theme and well known research workers and clinicians within the theme under discussion are invited to present main talks. In addition free communications are selected so that a programme is presented in which the sessions are scheduled in such a manner that both basic and clinical aspects of the topic are discussed.

The interest for the IVth Symposium in Nijmegen was extremely encouraging as were the reactions from the participants after the meeting. The organizers hope to continue this young tradition and the Vth Reinier de Graaf Symposium is scheduled again in Nijmegen in 1984.

The fact that Excerpta Medica has agreed to publish the proceedings is an additional and important stimulus to continue with the Reinier de Graaf Symposia on Ovarian Function.

Nijmegen

R. ROLLAND

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THE 'REINIER DE GRAAF LECTURE'

OVARIAN FOLLICULAR DEVELOPMENT FROM THE ONSET OF LUTEAL REGRESSION IN HUMANS AND SHEEP

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In humans and sheep, the recruitment of an ovarian follicle for preovulatory development and ovulation is thought to occur after the onset of luteal regression (1-3). The subsequent development of this follicle, into a preovulatory structure, is characterized by the entry of increasing quantities of oestrogen into its follicular fluid and a progressively greater output of oestrogen into ovarian venous blood (4-11). What remains obscure however is the relationship between the number of follicles with a capacity for oestrogen synthesis and the number which are recruited for preovulatory development. The reasons for this are at least three-fold. Firstly, there is a paucity of data pinpointing the stage of development when follicles develop the capacity for oestrogen synthesis. Secondly, there is only limited information on the relationship between the health of a follicle and its ability to synthesize oestrogen (7, 12-14), and, thirdly, there is a lack of quantitative data on the total numbers of follicles capable of being recruited for oestrogen synthesis.

The purpose of this review is to examine in humans and sheep: (a) some relationships between oestrogen and ovarian follicular development; (b) the stage of development when follicles aquire the capacity to synthesize oestrogen; and (c) the relationships between the number of healthy antral follicles and the number which are recruited for oestrogen synthesis after the onset of luteal regression.

OESTROGEN AND ANTRAL FOLLICULAR DEVELOPMENT

During the ovarian cycle, large numbers of antral follicles ($\geq$ 1 mm diameter) may be present in the ovaries of humans and sheep (sheep, 5-24 per ovary; humans, 6-46 per ovary). From a knowledge of the total population of granulosa cells in individual follicles of known diameter and the concentrations of oestradiol in follicular fluid, it can be shown that high intrafollicular levels of oestrogen (sheep $\geq$ 100 ng/ml; humans, $\geq$ 200 ng/ml)* are most commonly associated with follicles having the greatest number of granulosa cells with respect to follicular diameter, (Fig. 1a, b). And, that

* In ovine follicles without granulosa cells, the intrafollicular levels of oestradiol are always < 100 ng/ml. Likewise in human follicles without granulosa cells, the levels of oestradiol are < 200 ng/ml (15).

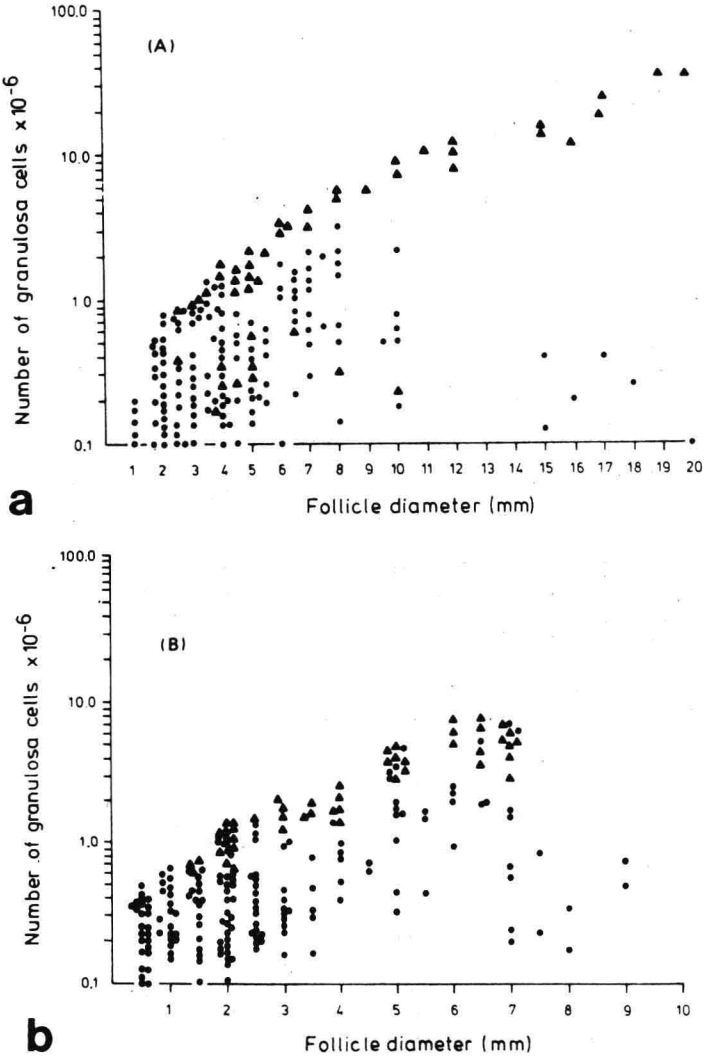

Figure 1. Relationship between the number of granulosa cells recovered from individual antral follicles and follicular diameter in (a) humans and (b) sheep. ● = follicles with < 200ng/ml oestradiol(a),< 100ng/ml oestradiol (b) ▲ = follicles with ⩾ 200ng/ml oestradiol(a),⩾ 100ng/ml oestradiol (b). All follicles were recovered from excised human and ovine ovaries during ovarian cycles which appeared to be normal. Excluded from the data are follicles recovered in the interval between the preovulatory LH surge and ovulation:oestradiol levels in healthy preovulatory follicles at this time are low (humans; ref. 16: sheep; ref. 10)

> 90% of follicles with less than 50% of the maximum number of granulosa cells present for a given follicular diameter (m.g.c./f.d.) have low levels of oestrogen (i.e. sheep, < 100 ng/ml; humans, < 200 ng/ml). Human follicles with ⩾ 200 ng/ml of oestradiol in antral fluid contain on average 16.5 ± 0.5 (n = 42) of their cells in the S-phase of mitosis whereas follicles with < 200 ng/ml of oestradiol have only 6.9 ± 0.5 (n = 74) of their cells in the S-phase (P < 0.01; ref. 17). Data from sheep, relating intrafollicular oestrogen to mitotic activity, are not available.

A significant finding of the data summarized in Fig. 1 is that, irrespective of the populations of granulosa cells, follicles < 2 mm in diameter contained low levels of oestradiol (sheep, < 100 ng/ml; humans, < 200 ng/ml). When the concentrations in larger diameter follicles exceeded 100-200 ng/ml they were between 300 and 500.000-times higher than those in the peripheral blood stream. It is reasonable to assume that these high levels reflect an increased capacity of a follicle for steroid synthesis rather than for sequestering steroid from the blood-supply. This interpretation is consistent with the in vitro findings on basal and FSH-stimulable oestrogen-synthetase activity in the membrana granulosa from both human and ovine ovaries (Fig. 2a,b). In follicles < 2 mm diameter, the capacity of granulosa cells for synthesizing oestrogen is low. As follicles with ⩾ 50% m.g.c./f.d. increase in diameter beyond 2 mm there is a concomitant increase in the levels of basal and/or FSH-stimulable oestrogen-synthetase activity in the membrana-granulosa. In large follicles (humans, ⩾ 12 mm diameter; sheep, ⩾ 5 mm diameter) with ⩾ 50% m.g.c./f.d., FSH appears no longer effective in stimulating oestrogen-synthetase activity above the levels already present in control cultures (Fig. 2). When granulosa cells were recovered from human or ovine follicles which were severely deficient in granulosa cells (i.e. follicles with < 50% m.g.c./f.d.), the levels of FSH-stimulable and/or basal oestrogen-synthetase activity (on a per cell basis) were, in most instances, low: < 75% of all such follicles (> 2 mm diameter; sheep, n = 26; humans, n = 102) contained granulosa cells which were incapable of generating more than 3 ng (sheep) or 10 ng (humans) of oestradiol per 10^6 cells per 48 h in vitro (Fig. 2, ref. 18). Moreover, from in vivo studies on ovine ovaries, it has been shown that > 98% of the follicles with < 50% m.g.c./f.d. (n = 57) were unable to generate intrafollicular levels of oestradiol ⩾ 100 ng/ml after exposure to PMSG (3).

Granulosa cells are probably not the only source of follicular oestradiol (2). Nevertheless the above results suggest that their capacity to generate oestradiol under in vitro conditions provides a useful biochemical marker for determining whether a follicle has a potential for further development.

In human follicles < 12 mm diameter, oestrogen-synthetase enzyme activity in isolated granulosa cells is stimulable by FSH but not HCG (19): presumably this specificity of gonadotrophin action also applies in the sheep although small healthy ovine follicles (1-4 mm diameter) have specific receptors on their granulosa cells for LH as well as FSH (Fig. 3, ref. 20). As ovine follicles enlarge to ⩾ 5 m in diameter, there is a 10- to 50-fold increase in the number of unoccupied HCG/LH receptors in the granulosa cells of follicles with ⩾ 50% m.g.c./f.d. compared to that

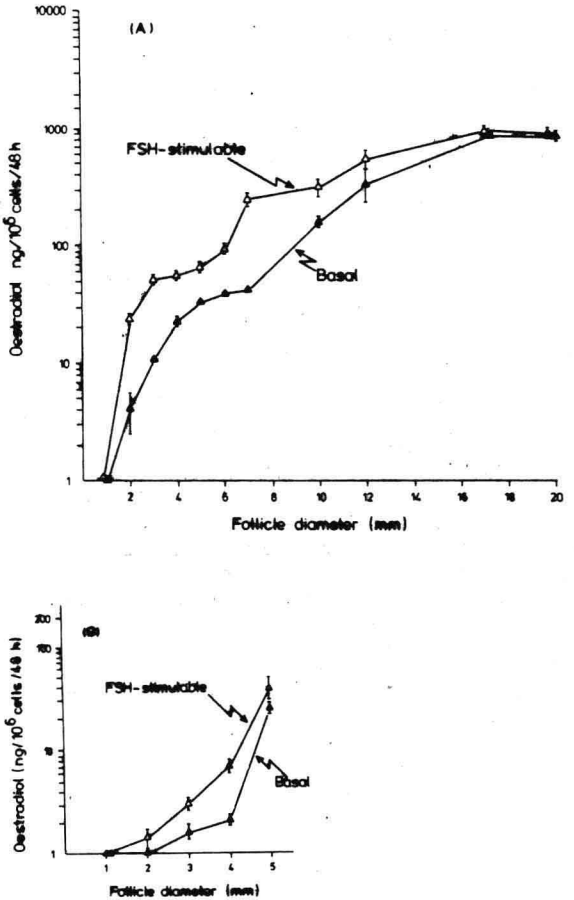

Figure 2. Basal (▲—▲) and FSH-stimulable (Δ—Δ) oestrogen-synthetase activity in granulosa cells of different-sized follicles from human (a) and ovine (b) ovaries (mean ± s.e.m.). All follicles contained at least 50% of the maximum number of recoverable granulosa cells with respect to follicular diameter (3,12). Granulosa cells ($5\text{-}2\times10^3$ 'live' cells/dish were cultured for 48 h in 1ml of 80% (v/v) Medium 199, Hepes buffer 20mM, Hanks' salts, antibiotics, 20% foetal calf serum (v/v) with either 1μg/ml androstenedione to assess basal activity or 1μg/ml androstenedione + 0.1μg/ml FSH (humans, NIH-FSH, LER 1951; sheep, NIH-FSH-S10) to assess FSH-stimulable activity. Oestradiol in the medium was measured after celite chromatography by radioimmunoassay (3,12).

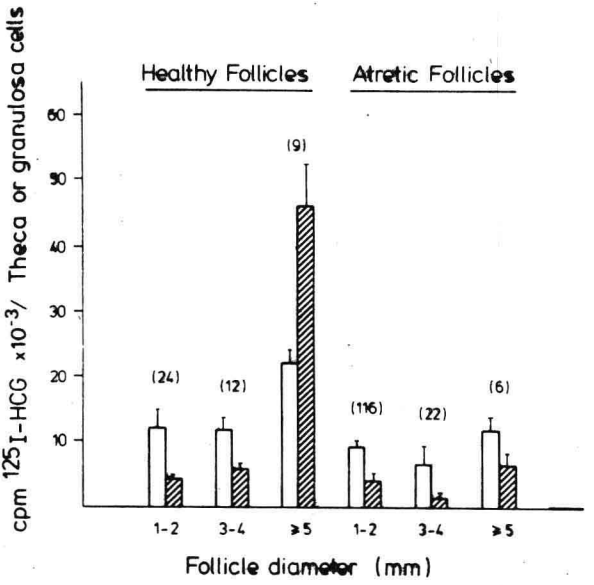

Figure 3. Evidence for the presence of specific HCG/LH receptors in ovine thecal tissue (white histogrammes) and granulosa cells (shaded histogrammes) of different-sized healthy or atretic follicles. Results are expressed as mean (+ s.e.m.) c.p.m I^{125} HCG bound per entire theca or per total population of granulosa cells in the different-sized follicles. Number of individual follicles examined are indicated in parentheses. Ovarian tissues were recovered from ewes during the oestrous cycle. Binding of isolated granulosa cells or homogenized theca was tested after $2x10^5$ cpm of I^{125} HCG (25μCi/μg) were added to the cells or tissue in 0.01 M phosphate buffer with 0.14 M NaCl, 5mM $MgCl_2$, 0.1 M sucrose and 0.1% ovalbumin (pH 7.2) and the mixture incubated at 37°C for 3 h. Non-specific binding was determined in the presence of unlabelled HCG at a concentration of 200 IU/ml. Specific binding was then calculated by subtraction of counts bound in the presence of excess unlabelled gonadotrophin from total counts bound. The capacity of ^{125}I-HCG to bind to receptor was pretested against an ovine luteal cell membrane preparation. Healthy follicle = one with a healthy-looking oocyte and ≥ 50% maximum number of granulosa cells for a given follicular diameter (m.g.c./f.d.). Atretic follicle = one with either a healthy or degenerating oocyte but with < 50% m.g.c./f.d. (21)

in similar-sized follicles with < 50% m.g.c./f.d. or that in small follicles (1-2 mm diameter) (Fig. 3, ref. 20). Although specific HCG/LH receptors have been found in human granulosa cells (22), the change in their number with respect to the health and size of the follicle is not known. In the rat, it has been reported, that HCG/LH is capable of stimulating oestrogen-synthetase activity in granulosa cells (23). However, the role (if any) of HCG/LH in stimulating oestrogen-synthetizing activity in the granulosa cells of large follicles from ovine (i.e. ⩾ 5 mm diameter) and human (i.e. ⩾ 12 mm diameter) ovaries remains to be determined.

Follicles with > 75% m.g.c./f.d. have, irrespective of follicular diameter (⩾1 mm), the greatest proportion of healthy-looking germinal-vesicle stage oocytes whereas follicles with < 26% m.g.c./f.d. have the lowest proportion (Table 1). These data probably overestimate the true incidence of recovering healthy oocytes since the extent and nature of the oocyte-cumulus cell

Table 1. Relationship between the percent of granulosa cells present in follicles of known diameter and the number of healthy-looking germinal vesicle (GV) stage oocytes

Number of Granulosa Cells (%)*	Number of healthy-looking GV-stage oocytes ÷ Total number of follicles examined (%)	
	Humans	Sheep
> 75%	33/41 (80.5)	36/42 (85.7)
51-75	39/62 (62.9)	22/36 (61.1)
26-50	39/85 (45.9)	26/56 (46.4)
< 26	32/120 (26.7)	9/40 (22.5)

* Number of granulosa cells (%) = (Total number of granulosa cells recoverable from a follicle of known diameter ÷ Maximum number of cells known to be present in a follicle of that diameter) x 100