MATURATION OF FOLLICULAR OOCYTES IN THE PIG AFTER INJECTION OF HUMAN CHORIONIC GONADOTROPHIN

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Summary. The sequence of morphological changes in the nuclei of follicular oocytes in the pig has been examined after a single injection of human chorionic gonadotrophin (HCG), given during pro-oestrus.

Oocytes remained in the germinal vesicle (dictyate) stage until 18 hr after injection of HCG. Chromatin condensation, followed by completion of prophase of the first maturation division, then occurred, and the chromosomes were arranged on the first metaphase spindle by 26 hr after HCG. From 26 until 34 hr after HCG, the chromosomes remained at first metaphase, but between 35 and 37 hr, anaphase, telophase and abstriction of the first polar body were completed. The great majority of eggs recovered from individual animals showed similar stages of maturation.

INTRODUCTION

In mammals, primary oocytes reach the diplotene stage of meiosis at approximately the time of birth (Brambell, 1956; Zuckermann, 1960). The germinal material is then arranged within a vesicular nucleus, the dictyate stage, and oocytes remain in this arrested state until shortly before the time of ovulation or follicular atresia. The stages of meiosis between diakinesis and metaphase of the second meiotic division occur, in most species, during the brief period between stimulation of the Graafian follicle by luteinizing hormone and ovulation.

The resumption of meiosis and ovulation can be induced in many immature or adult mammals by the injection of gonadotrophins. Moreover, the stage of meiosis at a particular time after a gonadotrophin injection can often be determined with considerable accuracy (see Edwards & Gates, 1959; Edwards, 1962).

In the pig, gonadotrophins have been used for the induction of ovulation (Tanabe, Warnick, Casida & Grummer, 1949; Du Mesnil du Buisson, 1954; Spalding, Berry & Moffit, 1955; Dziuk & Baker, 1962; Dziuk & Polge, 1965; Day, Neill, Oxenreider, Waite & Lasley, 1965). But although Spalding *et al.*

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(1955) described the nuclear changes in follicular oocytes at various intervals after the onset of oestrus, the timing and duration of individual meiotic stages following the injection of gonadotrophins has not been determined.

Knowledge of the timing of the successive phases of the maturation divisions in pig oocytes would have a useful application in a number of experiments. The results presented below are concerned with this aspect of ovarian response to human chorionic gonadotrophin (HCG), and have been obtained as a necessary preliminary to studies on various aspects of fertilization in the pig.

MATERIALS AND METHODS

Twenty-eight gilts, predominantly cross-bred Large White $\times$ Essex, were used in these studies. All weighed 200 to 275 lb, and had experienced one or more oestrous cycles. Each animal was injected intramuscularly with 500 i.u. HCG (Lutormone, Burroughs Wellcome) in 5 ml sterile saline during late pro-oestrus, this stage being indicated by the colour and swelling of the vulva. Recording the 1st day of oestrus as Day 0, all injections were made on Day 19 or 20 of the cycle, and it was confirmed that animals would not stand to the boar at this time. At exact intervals after injection, animals were stunned and bled, and the reproductive tract was removed.

In order to recover follicular eggs, the ovaries were separated from the tract and, using a syringe with 22 gauge needle, all follicles of 6 to 7 mm or greater diameter were carefully punctured and the contents aspirated and transferred to a watch glass containing saline. It was normally possible to see the individual cumulus masses with the naked eye and the eggs could be distinguished within these under a binocular microscope ($\times 12.5$). The cumulus cells were dispersed by addition of ovine hyaluronidase (Hyalase, Bengel Laboratories) dissolved in saline and the eggs gently dissected free of the majority of adhering cells. They were then transferred to a second watch glass of saline, and whole mounts prepared on microscope slides. These procedures were completed within 25 to 30 min of slaughter.

After fixing in 25% acetic alcohol and staining with 1% natural orcein in 45% acetic acid, a careful examination of the morphological changes in the nuclei of the follicular oocytes was made by phase-contrast microscopy. The following classification was adopted to cover the stages observed.

Germinal vesicle

This is the dictyate stage in which the form of the nucleus is unchanged from that seen in primary oocytes recovered at earlier stages of the oestrous cycle. The large nucleus is located eccentrically or peripherally and contains fine granular material. The nuclear membrane is intact, and the nuclear area is quite distinct. The chromatin strands are arranged in the form of a ring or horseshoe around a single large nucleolus, again eccentrically placed (Pl. 1, Figs. 1 and 2).

Prometaphase

This term has been used to cover the stage between late prophase and early metaphase. The appearance of the nucleus has changed considerably. The

PLATE I

Whole mount preparations of follicular oocytes stained with aceto-orcein showing stages of maturation. Phase-contrast photomicrographs. $\times 1200$.

Figs. 1 and 2. Germinal vesicle. Figs. 3 and 4. Prometaphase. Fig. 5. First metaphase. Fig. 6. First anaphase.

Whole mount preparations of follicular oocytes stained with aceto-orcein showing stages of maturation. Phase-contrast photomicrographs. $\times 1200$.

Fig. 7. First anaphase. Figs. 8 and 9. First telophase. Figs. 10, 11 and 12. Abstriction of first polar body.

nuclear membrane can only be detected as a very fine strand, or is indistinct, and the nuclear area is less conspicuous due to a loss of granular material. The chromatin is undergoing condensation into either a single lump or into smaller discrete fragments, and the nucleolus is no longer apparent (Pl. 1, Figs. 3 and 4).

First metaphase

There is no trace of the nuclear membrane. The chromosomes are formed and arranged in an orderly sequence on the equator of a bipolar spindle. The long axis of the spindle is orientated radially to the surface of the egg (Pl. 1, Fig. 5).

First anaphase

The chromosomes are seen to be migrating towards the poles of an elongated spindle (Pl. 1, Fig. 6 and Pl. 2, Fig. 7).

First telophase

The chromosomes have reached the polar regions of the spindle, and the mid-body of the spindle is now distinct. The first polar body is being formed, but is still clearly attached to the spindle (Pl. 2, Figs. 8 and 9).

Second metaphase

The first polar body has been abstricted from the egg (secondary oocyte), and the chromosomes of the latter are once more arranged on the equator of the spindle (Pl. 2, Figs. 10, 11 and 12).

RESULTS

Two hundred and ninety-seven follicular eggs were examined, and the sequence of nuclear changes at varying intervals after the HCG injection is shown in Table 1.

No change from the dictyate nucleus was observed until 16½ hr after an injection of HCG. At this stage, in one animal, the chromatin strands associated with the nucleolus were slightly fragmented in five of the eggs and yet they were still arranged essentially in the form of a ring or horseshoe. The nuclear membrane in these eggs was intact and showed a smooth outline. The remaining five eggs had normal dictyate nuclei, and all ten eggs were classified as being at the germinal vesicle stage. At 18½ hr after the injection of HCG the first clear indication of a resumption of meiotic activity was found in three of thirteen eggs. The chromatin had condensed into discrete fragments, and these three eggs were classified as being at prometaphase.

The transition from the germinal vesicle stage to prometaphase was seen in an increasing proportion of the eggs recovered from animals between 18½ and 21 hr after injections of HCG. The nuclear membranes were becoming irregularly shaped and less well-defined than in the germinal vesicle stage, and the chromatin was condensing into small lumps or fragments. However, in fourteen eggs examined at 20½ hr after injection, although the nuclear membrane exhibited changes characteristic of the transition from the germinal vesicle stage to

prometaphase, the chromatin was still organized as a ring in association with the nucleolus.

By 22 hr after HCG injection, all fifteen eggs recovered from one animal were clearly at prometaphase, and this stage was seen in eggs from three other animals recovered at times up to 26 hr after injections of HCG. The nuclear membrane was no longer visible in these eggs. At 26½ hr, the first distinct arrangement of

TABLE 1
THE TIME RELATIONSHIP OF THE MATURATION STAGES IN
FOLLICULAR OOCYTES TO AN INJECTION OF 500 I.U. HCG GIVEN
DURING PRO-OESTRUS

Interval from HCG injection to slaughter in individual animals (hr)	Stage of maturation						Total No. of follicular oocytes examined
	G.V.	P.M.	M ₁	A ₁	T ₁	M ₂	
14	4						4
16	12						12
16	8						8
16½	10						10
17	5						5
18½	10	3					13
18½	1	7					8
19	2	8					10
20	7	6					13
20½	14						14
21	2	9					11
22		15					15
24½		13					13
25		13					13
25½		7					7
26½		1	3				4
26½			3		3		6
27	2	2	12				16
27			13				13
28	3	5	3				11
28			14				14
29½			12				12
34	1		6	1			8
36	3	1	12				16
36			1	1	2	3	7
36	1			1	4	7	13
36						9	9
37½				1	2	9	12

Stages: G.V., germinal vesicle; P.M., prometaphase; M₁, first metaphase; A₁, first anaphase; T₁, first telophase; M₂, second metaphase.

chromosomes on the metaphase plate was seen in three of four eggs, and inspection of Table 1 shows a progressive change from prometaphase to the first metaphase, so that by 29 hr after HCG injection all eggs in one animal had reached this stage. At 26½ hr, in one animal, three of a total of six eggs had reached the first telophase; and in two other animals a small proportion of the eggs appeared to be somewhat retarded in development. These findings are discussed below.

No eggs were observed in the interval between 29½ and 34 hr after HCG injection, but it seemed probable that the chromosomes remained arranged at

first metaphase in the majority of eggs during this period. In six of eight eggs examined at 34 hr the chromosomes were still arranged at the equator, but in one egg they were migrating towards the poles of the spindle. Forty-five eggs were examined from four animals at 36 hr after injection of HCG, and in nineteen (42.2%) the first polar body had been abstricted. In eight further eggs (17.8%) the chromosomes were at anaphase or telophase of the first meiotic division, whilst in thirteen (28.9%) they were still arranged at metaphase. Again, a small proportion of eggs with germinal vesicles was recovered from these animals. Finally, twelve eggs were recovered from one animal at 37½ hr after HCG injection. Nine of these eggs showed abstricted first polar bodies, two were at late telophase, and one was at anaphase of the first meiotic division.

From these observations on the maturation of follicular oocytes after an injection of HCG, it was apparent that the majority conform quite closely to a

TABLE 2

THE APPROXIMATE DURATION OF THE MATURATION STAGES IN FOLLICULAR OOCYTES AFTER INJECTING ANIMALS IN PRO-OESTRUS WITH 500 I.U. HCG

<i>Stage of maturation</i>	<i>Interval from HCG injection (hr)</i>	<i>Approximate duration of stage (hr)</i>	<i>% of oocytes conforming to timetable</i>
Germinal vesicle	0 to 18	18	100
Germinal vesicle to prometaphase	18 to 22	4	100
Prometaphase	22 to 26	4	100
Metaphase 1	26 to 35	9	78.6
Anaphase 1, Telophase 1, and abstriction of first polar body	35 to 37	2	71.3

timetable of nuclear changes (Table 2). The longest single stage after HCG injection is that of the germinal vesicle or dictyate nucleus (18 hr), whereas after visible resumption of meiotic activity the longest transitional stage is that of first metaphase (8 to 10 hr).

DISCUSSION

These observations on the sequence of morphological changes in the nucleus of the follicular oocyte are in good agreement with the few observations recorded by Spalding *et al.* (1955) after the onset of spontaneous oestrus. Of the five stages examined by Spalding *et al.* from 5 to 48 hr after the onset of oestrus, three of the animals were killed at principal stages in the maturation process, i.e. 18, 26 and 36 hr after the onset of oestrus. It can be reasonably concluded from the present observations, therefore, that the timing of the stages of diakinesis, first metaphase and second metaphase after an injection of HCG given during pro-oestrus corresponds very closely to the timing of these changes after the onset of spontaneous oestrus. This would be in agreement with the concept of an endogenous release of luteinizing hormone at approximately the time of onset of oestrus. Further support for this concept comes from other studies

(Dziuk & Baker, 1962; Dziuk & Polge, 1965) in which ovulation was found to occur approximately 40 hr after an injection of HCG. A considerable number of observations on the timing of spontaneous ovulation in the pig record that this occurs approximately 36 hr after the onset of oestrus. The discrepancy of 4 hr could be attributed to failure to detect the onset of oestrus precisely.

It is clear that the process of chromatin condensation, and the sequence between first anaphase and extrusion of the first polar body are relatively short, whereas the interval between the end of chromatin condensation and termination of the first metaphase is relatively long. These findings are in agreement with the work of Odor (1955) and Mandl (1963) on the oocyte of the adult rat, and of Dziuk (1965) on the oocyte of the sheep.

The majority of eggs recovered from individual animals were closely synchronized in their maturation stages. There was, however, a small number of eggs which remained in the germinal vesicle stage even up to 36 hr after the injection of HCG and these were found in conjunction with eggs at later stages of development. These retarded eggs usually showed a very tight corona radiata which was apparently unaffected by hyaluronidase, and it is suggested that they were immature eggs that were unable to respond to the injected HCG. It is possible that they were recovered from follicles that were not destined to ovulate at the end of that oestrous cycle.

The three eggs recovered at 26½ hr after injection of HCG, showing telophase spindles, had probably reached this stage of maturation as a result of an endogenous release of luteinizing hormone. Although all animals were tested with a boar immediately before the HCG injection, there are instances in which an animal in oestrus may not react to the presence of a male in the short time allotted to testing. It is therefore considered probable that this animal was already in oestrus at the time of injection, and that the stage of egg maturation observed was not a result of the HCG.

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