

## Induction of Ovulation in Human Ovaries Perfused *in vitro*\*

E. Stähler, L. Spätling, H. D. Bethge, E. Daume, and R. Buchholz

Universitäts-Frauenklinik Marburg  
(Direktor: Prof. Dr. R. Buchholz)

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**Summary.** An *in vitro* ovulation could be induced and observed in four human ovaries with Graafian follicle. Perfusion was carried out by means of a semi-synthetic medium which was free from haemoglobin in a closed recirculating system. During the stimulation by means of HCG/HMG (after the II. IRP a total of 22.9 I.U. LH/ml and 3 I.U. FSH/ml) an activation of respiration and glycolysis by a factor of 2–3 up to the rupture of the follicle was observed. In this process 93% of the energy were released by the oxydative reduction of glucose and only about 7% by anaerobic glycolysis. 60–90 minutes before the rupture of the follicle an undulatory rise in the arterial hydrostatic pressure of up to 40 mmHg over the initial values was observed, although the outward conditions of perfusion were not altered, after the rupture a pressure drop to standard values. Apart from minor oscillations there was no increase in the intrafollicular pressure in spite of an increase in volume of the follicle, but only a decrease in pressure after the rupture of the follicle.

### Induktion von Ovulationen an *in vitro* perfundierten menschlichen Ovarien

**Zusammenfassung.** An vier menschlichen Ovarien konnte *in vitro* unter der Stimulation mit HCG/HMG eine Ovulation induziert und beobachtet werden. Die Ovarien wurden in einem rezirkulierenden System mit einem hämoglobinfreien, halbsynthetischen Medium perfundiert. Unter der Stimulation nahmen Atmung und Glykolyse bis zur Ruptur des Follikels um einen Faktor 2–3 zu. Dabei wurden aber —93% der freiwerdenden Energie aus dem aeroben Glucoseabbau gewonnen und nur —7% durch anaerobe Glykolyse. In der direkt präovulatorischen Phase konnte trotz Volumenzunahme des Follikels kein Anstieg des intrafollikulären hydrostatischen Druckes beobachtet werden. Der arterielle hydrostatische Druck dagegen zeigte 60–90 min zuvor einen wellenförmigen Anstieg bis zu 40 mmHg über den Normallevel, nach der Follikelruptur Abfall auf Ausgangswerte. Die Ergebnisse werden im Hinblick auf den Ovulationsmechanismus diskutiert.

### Introduction

The progress achieved in endocrinology during the last decades has led to a better understanding of the hormonal factors which bring about the cyclical changes in the ovary. Yet the mechanisms which cause the rupture of the follicle have still not been completely elucidated. Different

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causes were discussed: the phenomenon of a rapid increase in volume of the intrafollicular liquor during the preovulatory phase was said to result in an increase of the intrafollicular pressure, which in turn causes the rupture of the follicle (Thomson, 1919; Walton, Hammond, 1928; Brambell, 1928; Smith, Ketteringham, 1938; Hisaw, 1947; Burr, Davies, 1951; Rouget, 1858; Zachariae, Jensen, 1958). Possibly the increase in volume has to be attributed to various mechanisms. The depolymerization of the antral mucopolysaccharide by the hyaluronidasis leads to an increase in the colloid-osmotic pressure, which causes an influx of liquor and with it an increase in volume (Jensen, Zachariae, 1958; Zachariae, 1958; Zachariae, Jensen, 1958). Several investigations also point out that the permeability of the vessels (Burr, Davies 1951, Zachariae, 1958) and the permeability of the follicle membrane itself (Fitko, Stelmasiak, 1968) increases prior to the ovulation and that this is the reason for the influx of liquor into the follicle. Other authors gathered that changes within the vascular system were the cause for this process. Hyperaemia, engorgement and congestion of the ovarian blood vessels together with a rise in the arterial blood pressure were said to cause the rupture of the follicle (Clark, 1900; Heape, 1905; Keller, 1943; Pearson, 1944; Burr, Davies, 1951; Hisaw, 1947). It was possible in this connexion to establish a dependence between blood pressure and intrafollicular pressure (Landis, 1934; Espey, Lipner, 1963; Stähler, 1973). Even the lymphvessels could be a factor in the liquor production according to the investigations of Andersen (1926) and Burr, Davies (1951) (on ovaries of pig and cow). The liquor folliculi is reported to be a transudate of the theca blood-vessels (Simon, 1904) and in their compositions there is no appreciable difference between liquor and plasma (Lutwak-Mann, 1954; Caraviglios, Cilotti, 1957). As tests with water, which had previously been marked with tritium, show, there is a quick exchange of water molecules between the vascular compartment and the follicular antrum (Peckam, Kickhofer, 1959), and radioactive  $J^{131}$  applied i.v. appears in the follicle liquor after 15 minutes (Kaulla, Aikawa, Pettigrew, 1958). It was also maintained that the increase in the intrafollicular pressure is to be attributed to the contraction of smooth muscular tissue, which was indeed detected in the ovary by several authors (Guttmacher, Guttmacher, 1921; Thomson, 1919; Kraus, 1947; Rouget, 1858; Lipner, Maxwell, 1960; Okamura *et al.*, 1972). However, other groups of authors did not succeed in confirming this theory and the presence of smooth muscle-fibres in the ovary (Claesson, 1947; Espey, 1965; Espey, Slagter, Weymouth, Rondell, 1965)!

And yet spontaneous contractions of the ovaries were observed and measured with human and cat's ovaries (Palti, Freund, 1972; Rocereto *et al.*, 1969), and even in the ovaries of rabbits Espey, Lipner, 1963 could establish muscle-like contractions of the follicle. This does, of



course, point to the presence of smooth muscular fibres. However, more recent experiments revealed that, at least, in the case of rabbit and rat follicular growth and ripening take place without a measurable increase in the hydrostatic pressure (Blandau, Rumery, 1963; Espey, Lipner, 1963; Rondell, 1964). And, what is more, the quick increase in volume of the follicle shortly before the ovulation was even accompanied by a slight decrease of the intrafollicular pressure. This indicates that there must be additional mechanisms inducing the decomposition of the elastic elements of the follicle wall and there by enabling stigmatization and ovulation. As a possible cause an enzymatic digestion was assumed (Hartog, 1904; Schochet, 1916; Rugh, 1935; Moricard, Gothie, 1946; Kraus, 1947; Schubert, 1958; Jung, Kidess, 1959). But at the same time the possibility was discussed that the reduced haemorrhage within the area of stigmatization may cause a hypoxia leading to a necrosis (Clark, 1900; Blandau, Rumery, 1963; Wester, 1921; Nalbandov, 1961; Shettles, 1963; Espey, Lipner, 1963). One did also succeed in detecting numerous proteolytic enzymes in ovary and follicle (Cerletti, Zichella, 1961; Jung, Held, 1959; Reichert, 1962), which may play a role in the process of the fibrous decomposition in the follicle which is ready for ovulation. Espey (1969) was able to show that the fibroblasts in the follicles of rabbits protrude microvesicles shortly before the ovulation, the contents of which digest the elementary substance. The extensibility of the follicle wall does, at any rate, increase (enzymatically, biophysically, necrotically ?) during the preovulatory phase by a factor of 5-10 (Rondell, 1964, 1970). The investigations by Rodbard (1968) and Rondell (1970) into the biophysical connexions between pressure-volume, pressure-radius and the extensibility of the follicle wall reveal that changes in the follicle volume may take place without measurable changes in the hydrostatic pressure. However, they bring about an increase in the tension of the wall, strong enough to effect a rupture, if the tension of the follicular tissue is sufficiently low. The present paper is intended to contribute to the elucidation of the ovulatory process by a simultaneous measurement of some biochemical and biophysical parameters during *in vitro* induced ovulations.

### Material and Method

**Ovaries.** The ovaries were all taken from women with regular cycles, who had to subject to an abdominal extirpation of the whole organ because of a collum-ca I. At the time of the extirpation there existed Graafian follicles, and the ovaries were immediately stored in an iced medium. After the cannulation of the arteria ovarica under aseptical conditions the ovaries were flushed free from blood by means of a heparinized medium. Mean of age  $33.2 \pm 1.2$  years, mean of weight  $8.2 \pm 1.6$  g (w.w.). Period of "warm ischemia" several seconds, "cold ischemia" 12 minutes at most.

**Perfusion Technique.** Perfusion was carried out in a closed recirculating system at  $36.5^\circ\text{C}$  by means of a fully synthetic medium which was free from haemoglobin

and contained 6.5 g % bovine-serum albumin and which otherwise corresponded in its composition to the data of earlier investigations (Stähler, Huch, 1971; Stähler *et al.*, 1971; Stähler *et al.*, 1972). The rate of flow was 1.3–1.5 ml/min/g (w.w.); under these conditions we did not observe a pH-drop, and the production of lactate proceeded in a constant and linear way. The  $pO_2$ -value on the arterial side amounted to 715–720 mmHg. The perfusate was completely replaced every two hours.

**Stimulation.** When the revitalization is completed (duration appr. 20 min), a steady state is reached in the metabolic process. It was only after this state had been maintained for two hours that the stimulation was effected by means of gonadotropines: HCG (Primogonyl) according to the II IRP 12.9 I.U. LH/ml and HMG (Humegon) according to the II IRP 10.0 I.U. LH/ml and 3.0 I.U. FSH/ml (Daume, 1971). In the experiments the ovulation did, of course, not occur exactly within the same periods of time after the beginning of the stimulation. Differences in time between 1 and 3 hours were observed. For reasons of a better perspicuity only the joint last hours before the ovulation were comprised in the diagrammes.

**Analyses.** The  $O_2$ -absorption was determined by the arteriovenous difference between the partial  $O_2$ -pressures. The arterial pressure was measured immediately before the ovary by means of a Statham Db 23 pressure gauge; for the determination of the intrafollicular pressure (Statham Db 23) the probe, which was 0.5 mm thick, pointed and bent by nearly  $180^\circ$ , was laterally inserted far into the follicle, without injuring the follicle wall. pH,  $pO_2$ , arterial and intrafollicular hydrostatic pressure were continuously recorded on a multi-channel recorder (Hellige/Frbg.). All ovaries were histologically examined after the perfusion. Glucose was determined by means of the hexokinase-reaction, lactate and pyruvate by means of the LDH-reaction (Boehr).

## Results

The stimulation by means of gonadotropines (HCG/HMG) led to energy-releasing processes. Two of the main parameters for the determination of the metabolic activity are the  $O_2$ -absorption and the glucose-consumption; they are shown in Fig. 1. The glucose-consumption increased between steady state and the marked instant of the rupture of the follicle by a factor of 2.4 ( $p < 0.005$ ); the  $O_2$ -absorption increased by a factor of 3.0 ( $p < 0.02$ ) and remained at nearly the same level after the ovulation, while the glucose-absorption dropped during the same period of time ( $p < 0.10$ ) and showed an upward tendency afterwards. A good measure for a sufficient oxygenation of the isolated organ is the behaviour of the two metabolites lactate and pyruvate, the variations of which are shown in Fig. 2. Here, the stimulation led to a quick rise in the production of lactate, which up to the rupture of the follicle reached a factor of 3 ( $p < 0.005$ ). As was the case with the glucose-absorption, the lactate production fell clearly off after the rupture of the follicle ( $p < 0.01$ ) and rose again afterwards. Pyruvate, which in the steady state and without gonadotropic stimulation was always absorbed out of the perfusion medium by the ovary, was now, under the influence of HCG/HMG, actively delivered to the perfusion medium, and that with an increasing concentration ( $p < 0.025$ ). The behaviour of the released energy may be determined by means of the  $O_2$ -consumption and the glucose-

consumption. The result is shown in Fig. 3. It reveals that the whole released energy increases by a factor of about 3.3. The portion of energy derived from anaerobic glycolysis in it did, however, only amount to 7.1%, although this energy, too, rose by a factor of 2.2. This implies

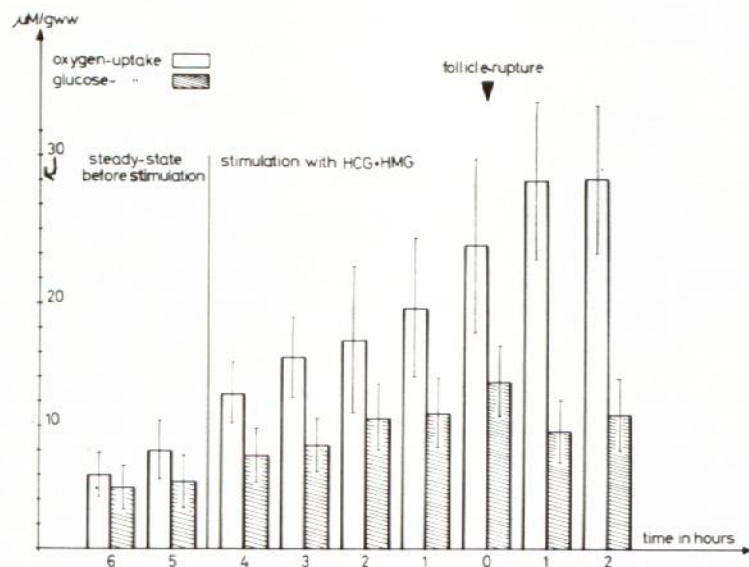


Fig. 1. Oxygen-uptake and glucose-consumption of four human ovaries perfused in vitro. Data in  $\mu$  Mol/g ovary [w.w.]/hour  $\bar{x} \pm SE$

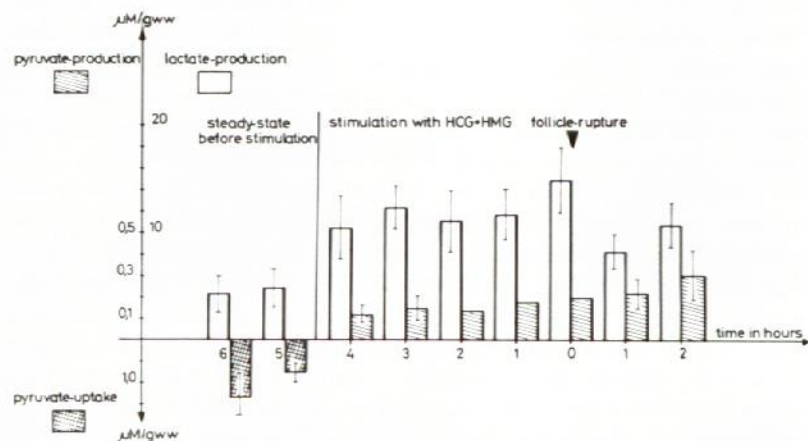


Fig. 2. The production of lactate and the response of pyruvate in four human ovaries perfused in vitro. Data in  $\mu$  Mol/g ovary [w.w.]/hour  $\bar{x} \pm SE$



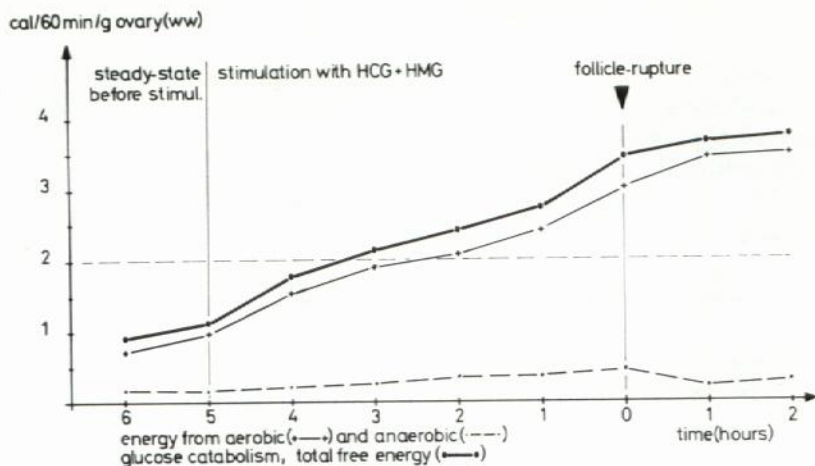


Fig. 3. The release of energy—Calculated from the oxygen-uptake and glucose-consumption. Data in cal/60 min/g ovary [w.w.]

that the main part of the energy was derived from the oxydative reduction of the glucose (increase by a factor of 2.9). Respiration as well as anaerobic glycolysis increased by the stimulation. Fig. 4 shows the changes of the arterial hydrostatic pressure without the outward conditions of perfusion being changed or vaso-active substances being added to the perfusion medium. As can clearly be seen the pressure does not remain constant on a certain level; there are pressure fluctuations with differing amplitude and time-lag. Approximately 1 to 1½ hours before the rupture of the follicle an undulatory rise of pressure up to a maximum of 40 mmHg over mean value was observed. Subsequent to the rupture of the follicle the pressure dropped again to the initial values. We did not observe this phenomenon with 8 other human ovaries of the same functional condition, in which no rupture of the follicle occurred during the perfusion, although there were also minor pressure fluctuations in each case (Stähler *et al.*, 1972). In spite of the increase in volume of the follicle we did not record a rise of the intrafollicular pressure (Fig. 5). Only insignificant oscillations were observed and, at the time of the ovulation, there was a rapid pressure drop, which did not, however, reach 0-values, for small remnants of liquor were left over. The macroscopic changes are shown in pictures I–VIII, which in two cases could be taken through the perfusion chamber. Cone-formation, increase in volume of the follicle and ovulation after stigmatization were basically the same in each of the four cases. The ovulation was no explosive process, but the clear, yellowish, slightly viscous liquor of the follicle ran off relatively slowly and little by little, as had already been observed in situ by Doyle (1951).

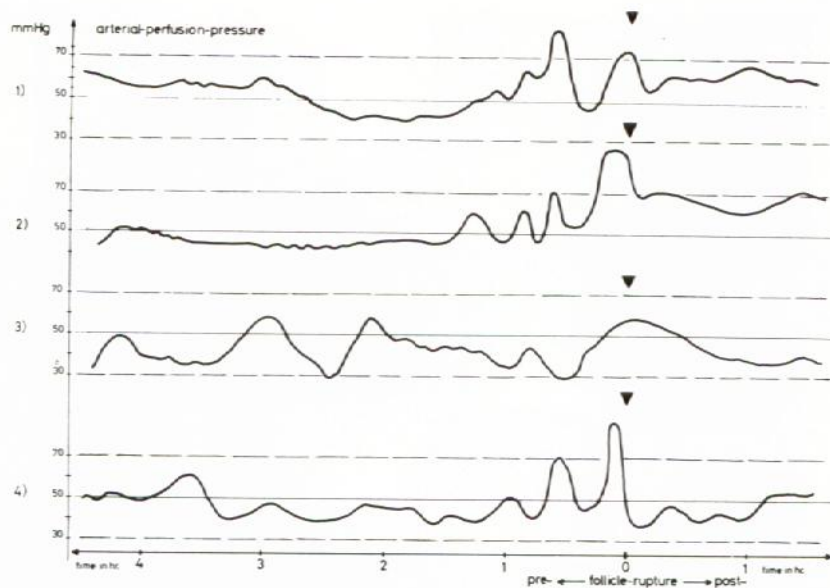


Fig. 4. The behaviour of the arterial hydrostatic pressure during the perfusion. The outward conditions of perfusion were not altered, data in mm Hg

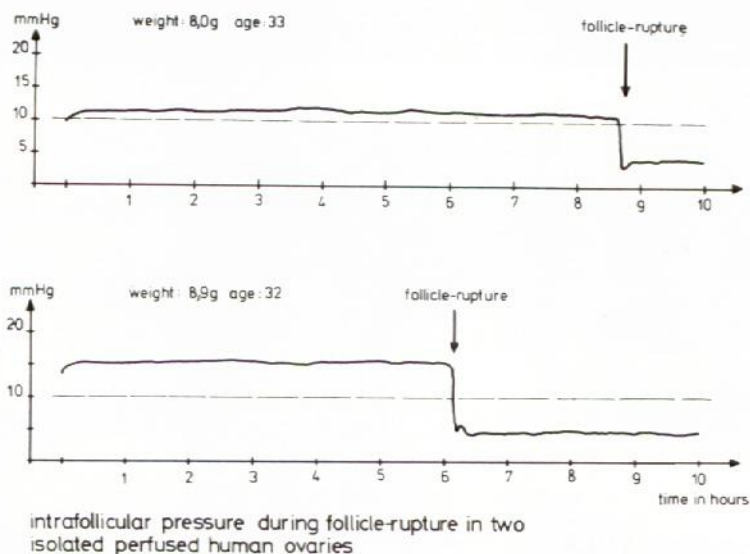
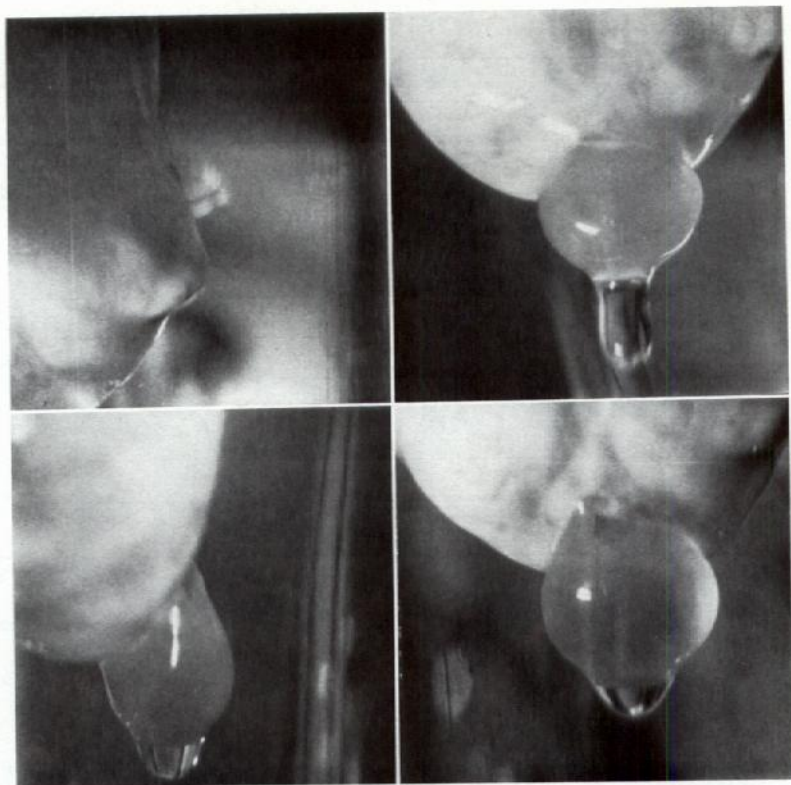


Fig. 5. The intrafollicular hydrostatic pressure during perfusion in the last hours before and after follicle-rupture, data in mmHg



I-IV

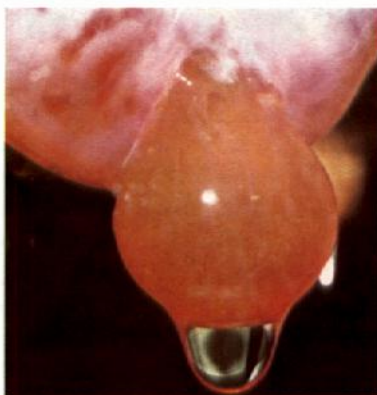


V

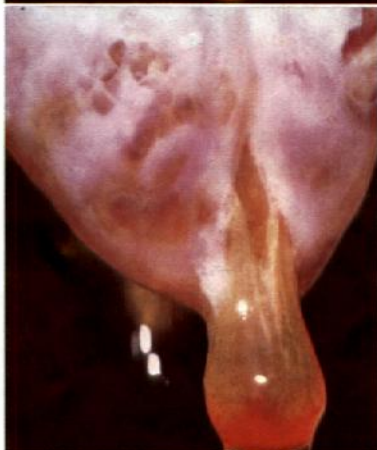
The pictures I-VIII were made through the organ-chamber. After cone-formation I, increase in volume of the follicle in 3-4 hours II, IV, VI. Ovulation V, VII, VIII after stigmatization-which is marked by an arrow-was no explosive process, the clear-yellowish, slightly viscous liquor ran off relatively slowly



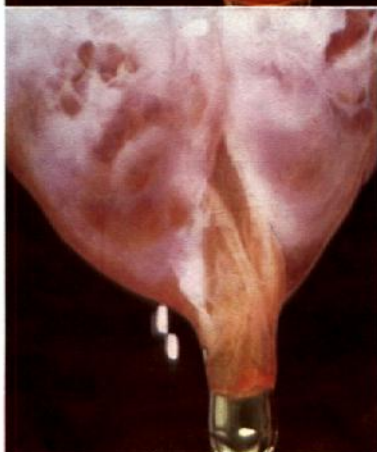
VI



VII



VIII



### Discussion

We have a detailed knowledge of the anatomy of the ovulation. (Blandau, 1967). However, the biochemical and biophysical changes could not yet completely be elucidated. Numerous investigations indicate that respiration and glycolysis are activated under the influence of gonadotropines. Under LH the  $O_2$ -absorption of isolated granulosa cells increased by a factor of up to 3.6 (Hamberger, Ahren, 1967; Hamberger, 1973). This corresponds in its order of magnitude to the increase established for the  $O_2$ -absorption, i.e. by a factor of 3. Ovulating fragments of rana pipiens ovaries consumed more  $O_2$  than did non-ovulating fragments and after the addition of pituitary extract an increasing  $O_2$ -absorption was observed (Rondell, Wright, 1957). Bovine ovaries with Graafian follicles showed a considerably higher  $O_2$ -consumption than ovaries without follicles (Stähler, Huch, 1971). If glycolysis, the tricarboxylic acid cycle or respiration are blocked, the ovulation does not take place. (Rondell, Wright, 1958). But LH does also increase the glycolytical activity, for with the ovaries of rats (Armstrong, Greep, 1962; Armstrong, 1962; Ahren, Kostyo, 1963; Armstrong, Kilpatrick, Greep, 1963; Armstrong, 1963; Hamberger, Ahren, 1967; Ahren *et al.*, 1968; Ahren, Hamberger, Rubinstein, 1969; Perklev, Berglund, Ahren, 1971) as well as with human ovaries (Stähler *et al.*, 1972) an increased production of lactate and an increased absorption of glucose was observed under the influence of LH. Thus both, respiration and glycolysis are activated. In the present investigations this activation amounted to a factor of 2-3, where about 93% of the released energy were, however, provided by way of the oxydative reduction of glucose. The simultaneous production of pyruvate with an increasing concentration points to a sufficient oxygenation of the tissue and the massive stimulation of the glycolysis. In earlier investigations Landis (1934) and Espey, Lipner (1963) were able to prove that the increase in the hydrostatic pressure in the arterial vascular system results in an increase in the intrafollicular pressure. These findings could be confirmed by us on human and bovine ovaries (Stähler, 1973). The undulatory pressure increase in the arterial vascular system prior to the rupture of the follicle (Fig. 4) indicates that possibly endogenous, vessel-specific mechanisms are involved in the process of ovulation. According to the hypothesis of Starling (1895) the increase in the hydrostatic pressure with constant colloid-osmotic pressure corresponds to a rise in the effective filtration pressure. Owing to this pressure increase more liquor leaves the vascular region than enters it again, which results in a swelling of the tissue and thus above all of the follicle. And, as was shown by the investigations of Jacobowitz, Wallach (1967), numerous adrenergic and cholinergic nerve fibres can be detected in the

human ovary, which are mainly grouped around the blood vessels. This does also indicate that the tonisation of the vascular system is an important factor in the process of ovulation. In spite of a quick increase in volume shortly before the ovulation there was no increase in the intra-follicular pressure (Fig. 5); this phenomenon had already been observed by Blandau, Rumery (1963), Espey, Lipner (1963) and Rondell (1964) with rats and rabbits. The extensibility of the follicle wall considerably increases towards the ovulation owing to a decomposition of the fibrous structure (Rondell, 1964; Rondell, 1970); this implies that at this time an increase in volume of the follicle is not necessarily accompanied by a measurable rise in the intrafollicular pressure (Rodbard, 1968; Rondell, 1970). If the phase of preovulatory decomposition of the connective tissue of the follicle has not yet been reached, every increase in liquor within the follicle will also lead to an increase in pressure, as was shown by Rondell (1964). Our findings confirm these investigations. The increase in the arterial pressure as well as the considerably increased production of lactate do both point to a change in the field of microcirculation. In addition, there is also a decrease in the production of lactate again after the rupture of the follicle together with the decrease in the arterial pressure to the initial values. It is also possible that there is only a local disturbance of the microcirculation with a subsequent hypoxia in the area of the follicle, what by way of necrotic changes could induce a decomposition of the follicle wall, as already assumed by other authors (Clark, 1900; Blandau, Rumery, 1963; Wester, 1921; Blandau, 1955; Nalbandov, 1961; Shettles, 1963). Whether or not spontaneous contractions of the ovaries, which have already been observed with human ovaries too (Palti, Freund, 1972), have a causal influence on the undulatory increase in the hydrostatic pressure in the arterial vascular system, would have to be determined by means of additional intraovarian measurements of pressure. The great metabolic activity of the generative active tissue of the ovary is revealed in the released energy, which at the time of ovulation reaches values of 3-4 cal/60 min/g of ovary, whereas e.g. for normal female persons (70 kg) only values of 0.79 cal/60 min g of body-weight were determined. (Wiss. Tabellen, Documanta-Geigy, 1960). Ovulation is an energy-consuming process.

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Dr. med. E. Stähler  
Universitäts-Frauenklinik  
D-3550 Marburg a.d. Lahn  
Federal Republic of Germany

