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Endocrine Changes in the Pig During Late Pregnancy, Parturition and Lactation [1]

DAVID M. BALDWIN[2] AND GEORGE H. STABENFELDT

Department of Reproduction, School of Veterinary Medicine, University of California, Davis 95616

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Peripheral plasmal levels of progestins, corticosteroids, and estrogens were determined in gilts beginning approximately 2 weeks prior to and at various intervals during parturition, as well as for up to 37 days postpartum in Yorkshire-Hampshire cross pigs. In addition, the same steroids were determined in 2 animals which aborted due to leptospirosis infection. Blood was obtained via a catheter chronically implanted in the lesser saphenous vein.

Progestins declined slowly in late gestation from about 15 ng/ml plasma until about 2 days prior to parturition, when levels decreased rapidly to about 3-4 ng/ml at delivery. A further decline in progestins was noted within 24 h after delivery with levels remaining low for up to 37 days postpartum. Progestin levels did not change during delivery. Corticosteroids, although variable in concentration, increased significantly 24 h prior to and during parturition. Estrogens increased continuously from 11 days prepartum (2 ng/ml) to 3 days prepartum (6.4 ng/ml), remaining constant through parturition with a precipitious decline observed by 24 h postpartum (2.5 ng/ml). Levels remained low (0.2 ng/ml) for up to 37 days after delivery.

In the 2 animals that aborted, progestin and corticosteroid patterns were somewhat similar to those noted for animals undergoing normal delivery, while estrogen levels differed from normal patterns in that estrogens declined in one animal and were low with a slight increase in the other animal prior to delivery. Although the number of observations is limited, it appears that premature delivery in the pig during late gestation due to disease processes is not accompanied by completely normal endocrine preparatory changes (estrogens in particular). This may be due to the fact that while the female may be able to respond in a normal manner to premature induction of labor, porcine fetuses appear to be immunologically incompetent (as compared to bovine and ovine fetuses) with fetal death the main result of exposure to stressor agents. Thus delivery may be preceded by fetal death which does not allow for the initiation of normal endocrine patterns.

Endocrine changes occurring during the onset of parturition have been studied in various mammalian species. The decline in peripheral plasma levels of progesterone in most species, e.g. rat, rabbit, hamster, goat, ewe and cow (see Davies and Ryan, 1972; Thorburn *et al.*, 1972 for references), regardless of an ovarian or placental origin, seems to be an important prelude to parturition. An exception is seen in humans (Llauró, *et al.*, 1968; Yannone, *et al.*, 1968) and subhuman primates (Stabenfeldt and Hendrickx, 1973) in which progesterone falls during or after delivery. While the importance of corticosteroids in the mother is not known, it is clear that fetal adrenals in cattle (Kennedy *et al.*, 1957) and sheep (Drost and Holm, 1968) are necessary for the initiation of labor. The rise in estrogens observed in a number of species prior to parturition may be important for the enhancement of uterine contractility as well as for the initiation of production of a smooth muscle stimulant, prostaglandin $F_{2\alpha}$, as suggested by Challis *et al.* 1972.

Several recent studies have reported on endocrine changes in the pig at term (Shearer *et al.*, 1972; Molokwu and Wagner, 1973; Killian *et al.*, 1973; Edqvist *et al.*, 1974). The current study extends the findings in the pig through additional information on endocrine

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[2] Present address: Department of Physiology, University of Cincinnati Medical College, Cincinnati, Ohio 45219.

changes during parturition. Data were also obtained concerning endocrine changes during premature delivery (abortion) during late gestation as well as during lactation.

MATERIALS AND METHODS

Animals

All animals used in this study were primiparous Yorkshire X Hampshire cross pigs. Average breeding age was 183 days at an average weight of 215 lb. The mean gestation length was 113 days (range, 110-115). The average litter size was 9.0 ± 1.2 (*m* ± SEM). Eighteen gilts were cannulated from which complete data were obtained for 10 animals.

Cannulation

Animals were cannulated approximately 2 weeks prior to expected delivery. Pregnant gilts were injected im with atropine sulfate (Lilly) 30 min before anesthesia at a dosage of 2 mg/100 lb body weight. The animals were then anesthetized by rapid injection of Surital (0.5 g/100 lb body weight, Parke-Davis) via the ear vein, followed by maintenance of anesthesia by use of Fluothane (Ayerst) gas in a closed circuit system at a rate of 4-6 ml Fluothane/hr/100 lb body weight.

The lesser saphenous vein was exposed and cannulated with sterile silastic tubing (Dow Corning, French size 6). The catheter was inserted 12-14 inches into the vein and secured at the site of entry by a suture previously attached to it with Silastic Medical Adhesive, Type A (Dow Corning). The remaining catheter was "threaded" subcutaneously along the midline of the rump and back by use of a stainless steel 12 inches, 8-gauge trochar. The catheter was then brought to the skin surface along the midline of the back approximately 4-6 inches anterior to the base of the tail. The exposed end of the catheter was fitted with a luer lock adapter and 3-way valve, both of which were sutured to the animal's back and kept clean by covering with several pieces of gauze and tape. The catheter was then filled with heparinized saline until blood was removed. Animals were maintained on antibiotics for three days postsurgery. Each sow was isolated in a farrowing pen and fed twice daily with water available *ad libitum*.

Bleeding

Daily blood samples were taken without restraint between 9:00 and 11:30 A.M. Once milk secretion was noticed, samples were taken every 6 h through the completion of parturition. The interval from milk secretion to time of parturition varied from 12-72 h. Samples were obtained from some animals at the start of farrowing, during and immediately after farrowing. After collection, the blood was immediately chilled, centrifuged and the plasma stored at −20°C until assayed.

Steroid Analysis

Total progestins were extracted from 0.5 ml of plasma with petroleum ether (nanograde, Mallinckrodt) and assayed using a competitive protein-binding radioassay (CPBR) technique (Baldwin and Stabenfeldt, 1974) similar to that described by Bassett and Hinks (1969). All samples were assayed against a progesterone standard. Procedural losses were adjusted for by the addition of tritiated progesterone.

Total corticosteroids were isolated by first extracting the plasma (0.1 ml) with petroleum ether to remove progestins and then with absolute ethanol to extract the corticosteroids. An aliquot of the ethanol extract was assayed using the CPBR procedure described for progestins (Baldwin and Stabenfeldt, 1974). This procedure measures both cortisol and corticosterone, but as shown recently by Bottoms *et al.* (1972) the major corticosteroid in the pig is cortisol. All samples were assayed against a cortisol standard.

Total estrogens were determined by radioimmunoassay (Lasley *et al.*, 1974) using a modification of the technique described by Tillson *et al.* (1970) utilizing an estradiol-17β standard curve. Unbound and bound hormone were separated by the use of column chromatography (Sephadex G-25, medium).

Diagnostic Procedures

Two animals delivered prematurely during the experiment. The cause of the abortion was determined to be due to infection by *Leptospira pomona*. The diagnostic procedures included the determination of leptospira titers in the gilts by use of an agglutination-lysis test utilizing live leptospira antigen.

RESULTS

Figure 1 illustrates peripheral plasma levels of total progestins recorded during late pregnancy, parturition, and early lactation. Progestins slowly declined from about 15 ng/ml plasma at about 100 days of gestation to approximately 10 ng/ml at 2 days (day 111 of gestation) prior to delivery. Progestin levels then declined more rapidly reaching an average value of 3.5 ng/ml at delivery. A further decline to 1.3 ng/ml was observed within 24 h of delivery with progestin levels averaging slightly greater than 1 ng/ml through day 37 postpartum. Progestin levels did not decrease during the farrowing process as shown in Fig. 1. One gilt had an unusual progestin pattern during farrowing in that progestin levels were 2.6 ng/ml at the start of labor, 16.7 ng/ml during labor, and 7.3 ng/ml at the finish of

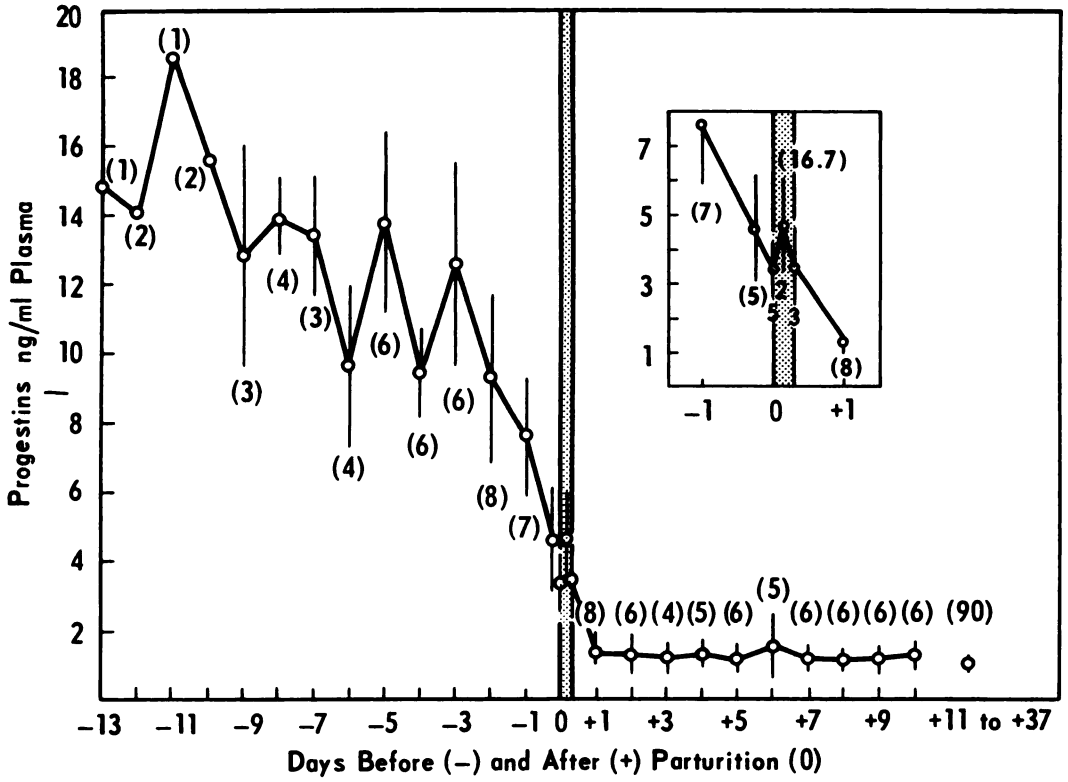

FIG. 1. Total peripheral plasma progestins ($m \pm$ SEM) in the pig before, during, and after parturition. The shaded area indicates the time of farrowing, and the numbers in parentheses represent the number of animals sampled. The insert (upper right) presents progestin changes observed at parturition. The high progestin value (16.7 ng/ml plasma) observed in one pig during labor was not included in the average.

labor. The progestin value in this animal was 1.1 ng/ml plasma by 24 h postpartum.

Total corticosteroids during the pre- and postpartum periods are shown in Fig. 2. As shown by the large standard error of the means, individual variation was large. Using an analysis of variance (repeated sampling) and Duncan's multiple range test of significance, mean values for days 3, 2 and 1 prepartum, day 0 and day 1 postpartum were compared. Corticoid levels were significantly lower on days 3 and 2 postpartum ($P < 0.05$) and day 1 postpartum ($P < 0.01$) as compared to levels 24 h prior to delivery (day 1 prepartum) and at parturition (day 0). Corticosteroid levels, which averaged about 9 μg/100 ml plasma at the start and during farrowing, were approximately 4 μg/100 ml plasma at the end of delivery. From days 2–37 postpartum, corticosteroid levels varied randomly from 2.5–7 μg/100 ml plasma.

Total estrogen patterns are shown in Fig. 3. Estrogen levels increased continuously from 11 days prepartum (2 ng/ml plasma) to day 3 prepartum (6.4 ng/ml plasma). Concentrations remained constant through farrowing, declining to 2.5 ng/ml by 24 h postpartum and averaging 0.2 ng/ml for 3 to 37 days postpartum. Estrogens began to decline at the end of farrowing as compared to those observed at the start or during labor, with a precipitous decline occurring over the next 24 h (see insert, Fig. 3). Considerable individual variation was noted in estrogen levels in that the peak values in individual animals ranged from about 3.5 to about 9.0 ng/ml plasma.

Several animals aborted during the experiment due to an outbreak of leptospirosis.

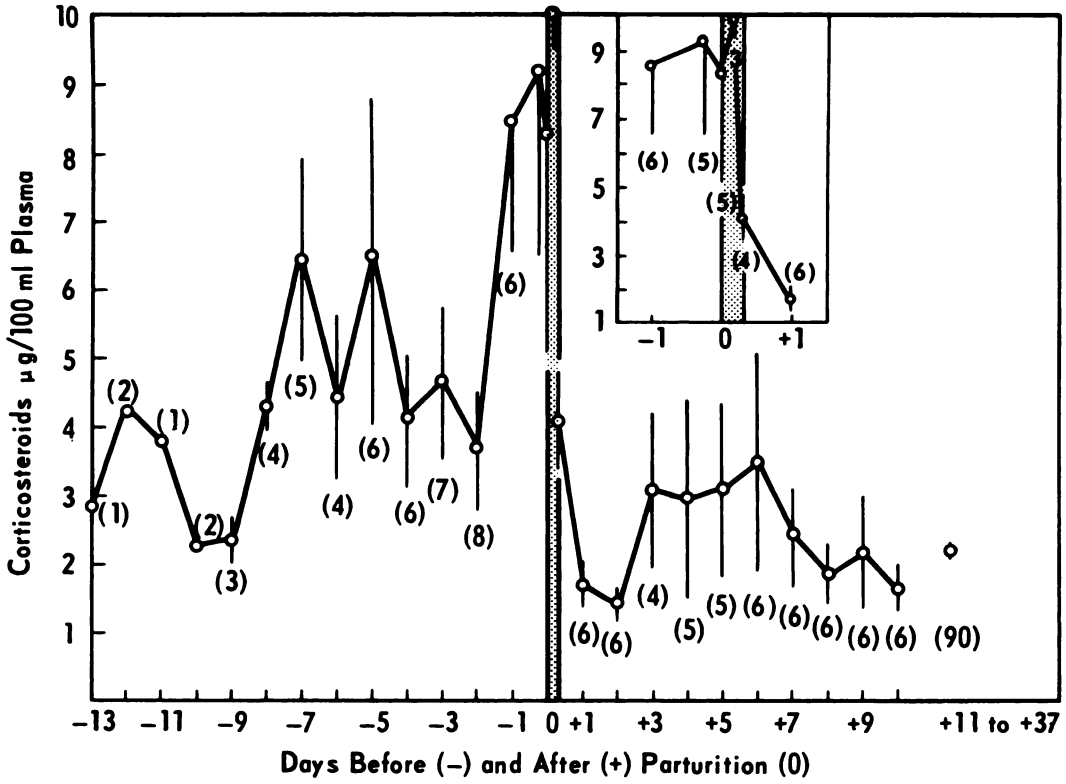

FIG. 2. Total peripheral plasma corticosteroids ($m \pm$ SEM) in the pig before, during and after parturition. The shaded area indicates the time of farrowing, and the numbers in parentheses represent the number of animals sampled. The insert (upper right) presents corticosteroid changes observed at parturition.

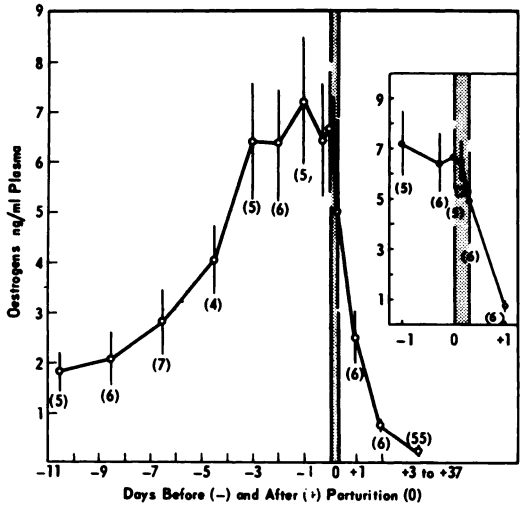

FIG. 3. Total peripheral plasma estrogens ($m \pm$ SEM) in the pig before, during, and after parturition. The shaded area indicates the time of farrowing, and the numbers in parentheses represent the number of animals sampled. The insert (upper right) presents estrogen changes observed at parturition.

Data were obtained from two of these animals which aborted dead fetuses on days 102 and 110 of gestation. Sampling began early in both of these animals because of the presence of milk in the mammae. Fig. 4 presents the data from gilt 182 which aborted on day 110. Both the progestin and corticosteroid patterns were similar to those observed in animals undergoing normal delivery in that progestin levels began a final decline 2–3 days before delivery, while corticosteroid levels increased prior to and declined after delivery. The estrogen changes were not similar to the normal pattern in that a continuous decline from 2 ng/ml plasma began about 3

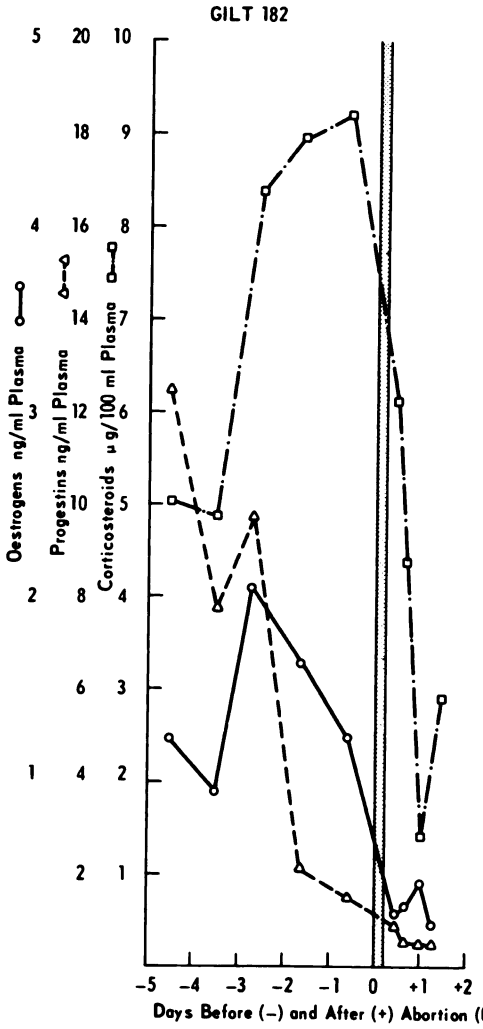

FIG. 4. Total peripheral plasma progestins, corticosteroids, and estrogens in one gilt which aborted at 110 days of gestation. The shaded area represents the time that abortion occurred.

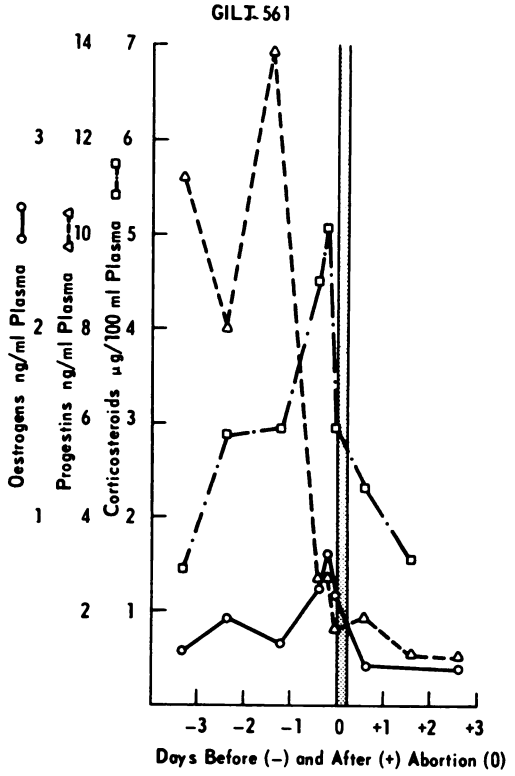

FIG. 5. Total peripheral plasma progestins, corticosteroids, and estrogens in one gilt which aborted at 102 days of gestation. The shaded area represents the time that abortion occurred.

days prior to abortion. The endocrine patterns in the gilt 561 which aborted on day 102 of gestation are shown in Fig. 5. Again, a progestin decline was noted although it occurred during the last 24 h prepartum. Corticosteroid levels which rose prior to abortion were decreasing at the time of delivery. Estrogen levels were low several days prior to abortion (0.6–0.8 ng/ml), increased 2-fold immediately prior to delivery, and then declined to about 0.4 ng/ml post-abortion.

DISCUSSION

The general decline in progestins observed in this study prior to parturition is in agreement with those of Molokwu and Wagner (1973) and Killian *et al.* (1973). It is apparent that the final decline of progestins begins about 2 days prior to delivery. The pig, however, does not undergo a complete withdrawal of progesterone prior to delivery (progestin levels 2–4 ng/ml) with a final withdrawal not apparent until a few hours postdelivery (1.0 ng/ml plasma, or less). In the present study, progestin levels were unchanged during farrowing in those animals that were sampled at the beginning of, during, and immediately after parturition. The pig thus is much like the ewe in that complete withdrawal of progestins does not occur prior

to delivery (Stabenfeldt *et al.*, 1972). It is apparent that the decline in progestin levels observed in the pig prior to delivery is sufficient to relieve the "progesterone block" which allows the onset of myometrial contractions and subsequent delivery.

The rise in total estrogens observed the last week of gestation in the present study is in general agreement with the findings of Molokwu and Wagner (1973), Robertson and King (1974), although estrogen levels were generally higher in our study. Our findings agree closely with those of Edqvist *et al.* (1974), both from the point of view of pattern and quantity. It is of interest that the cow, sow and ewe all experience a significant increase in plasma estrogens during the latter part of gestation. The estrogen increase begins about 3 weeks prior to term in the cow (Edqvist *et al.*, 1973; Robertson, 1974), 1 week prior to term in the sow or gilt (Molokwu and Wagner, 1973; Robertson and King, 1974) and 1 to 2 days before delivery in the ewe (Challis, 1971; Bedford *et al.*, 1972; Thorburn *et al.*, 1972; Robertson and Smeaton, 1973). The importance of the porcine feto-placental unit in the production of estrogen has been shown by the work of Bowerman *et al.* (1964) and Rombauts (1964) who reported findings that the placenta produced estrogens, and by Fèvre (1970) who demonstrated the involvement of the fetus in the production of estrone. Further, it was shown that neither ovariectomy nor hypophysectomy (Fèvre *et al.*, 1968) nor adrenalectomy (Fèvre *et al.*, 1972) of the pregnant sow influenced estrogen production. Our estrogen data obtained during farrowing support the concept that the feto-placental unit is important for estrogen production in that a decrease in estrogen concentration was first noted immediately after parturition (see Fig. 3).

It may be that the rise in estrogens in the cow, sow and ewe is associated with fetal maturity. This may have some relevance concerning the induction of parturition with glucocorticoids, both from the view of fetal maturity and ease of induction of labor. Cows are progressively easier to induce as term approaches, particularly during the last 2 weeks of gestation (Adams and Wagner, 1970). Bosc (1972) found that induction of labor with glucocorticoids was easier when treatment was begun on day 144 of gestation (as compared to day 142) at a time when estrogen production begins to rise in the ewe. From this it might be expected that greatest success for induction of labor and fetal viability in pigs would occur if treatment began no sooner than 7 days prepartum, a time when estrogen levels are beginning to increase.

The significance of the role of corticosteroids in maternal blood at parturition remains obscure. It is possible that corticoids alter the binding capacity of the uterus for progesterone by direct competition for binding sites or through modification of the receptors (Ryan, 1971). It may well be, as suggested by Molokwu and Wagner (1973), that the elevated maternal levels at delivery are a reflection of stress. The possibility exists, however, that this stress response is an integral part of the delivery process.

The progestin and corticosteroid levels in the 2 gilts which aborted were somewhat similar to those observed at term. The lack of a final estrogen surge is probably due to the fact that abortion occurred prior to a final maturation of the feto-placental unit. In cows, premature delivery of the fetus during late gestation usually results in delivery of a live fetus regardless of whether the labor was induced by pharmacological means (glucocorticoids) or through stress of the fetus by infectious agents. In addition, this premature delivery is characterized by endocrine changes that are similar to those observed in conjunction with normal term delivery, i.e., the bovine fetus is able to effect its delivery through the stimulation of normal parturitional processes (Edqvist *et al.*, 1972; Osburn *et al.*, 1969). Pig fetuses appear not to survive exposure to infectious agents as well as the bovine fetus, and thus premature delivery of porcine fetuses, even in late gestation, may

involve first death and then expulsion of the fetuses.

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