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Circulating Thyrotropin in the Ovine Fetus: Evidence for Pulsatile Release and the Effect of Hypothermia *in Utero*

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ABSTRACT. The secretion of thyrotropin (TSH) has been investigated in the chronically catheterized ovine fetus (term 145–150 days). Forty-two random plasma samples from 25 fetuses (86–149 days of gestation) were measured for TSH concentrations by radioimmunoassay. Plasma TSH concentrations were highest in the youngest fetuses [86–110 days, 3.9 ± (SD) 5.5 μU/ml, $n = 13$]. Thereafter TSH concentrations declined to 0.4 ± 0.6 μU/ml ($n = 13$, $p < 0.05$) at 130–150 days of gestation. However, serial sampling at 15–20 min intervals for 180 min from 14 individual fetuses (91–139 days) showed that TSH was secreted in a markedly exaggerated pulsatile manner compared to that observed after birth. The mean amplitude of TSH pulses fell ($p < 0.005$) from 5.9 ± 8.1 μU/ml in the fetuses to 2.1 ± 1.1 μU/ml in five neonatal lambs (6–22 days) and to 1.5 ± 0.4 μU/ml in three adult nonpregnant ewes. The mean pulse frequency for the 14 fetuses was 0.7 ± 0.3 pulses/h and was reduced ($p < 0.001$) to 0.3 ± 0.1 pulses/h in lambs and to 0.3 ± 0.1 pulses/h in the ewes. In the neonate, hypothermia is a potent stimulus to TSH release. To examine the ontogeny of this response, the temperature of the fetus *in utero* (106–127 days of gestation) was lowered by circulating water (14–18° C) at either a fast or slow rate through a coil placed either externally around the fetus or internally in the fetal esophagus and stomach. The fetuses were cooled for a period of 1 h during which fetal samples were obtained. In eight fetuses (106–127 days), the mean plasma TSH concentration rose ($p < 0.001$) from 1.5 ± 1.6 μU/ml to 6.7 ± 2.1 μU/ml during 1 h of fast external cooling (maximum fetal temperature fall 3.5° C). Plasma TSH values also rose ($p < 0.02$) during fast internal cooling (maximum temperature fall 1.9° C) from 1.4 ± 1.9 to 5.9 ± 8.2 μU/ml ($n = 5$, 116–132 days). A slower rate of cooling either externally (temperature fall 1.1° C) or internally (temperature fall 0.9° C) induced a variable response. The marked pulsatility of fetal TSH secretion which decreases after birth is postulated to be a consequence of immature negative feedback by thyroid hormones on fetal TSH release. The presence of a TSH response to hypothermia from 0.7 gestation is evidence that neuroendocrine mechanisms mediating TSH release and hypothalamic thermoregulatory responses have differentiated by this age. (*Pediatr Res* 19: 208–212, 1985)

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Abbreviations

TSH, thyrotropin
TRF, thyrotropin releasing factor
T_3, triiodothyronine
PBS, phosphate buffered saline

While the ontogenesis of the hypothalamic-pituitary thyroid axis has been extensively studied in several species (6), there are limited data regarding the ontogenesis of the secretion of TSH in the fetus. Observations have been largely restricted to the measurement of plasma TSH concentrations in the fetus following either fetal thyroidectomy, hypophysectomy, or pituitary stalk section (27), and to the demonstration of TSH responses to TRF in the fetal and newborn lamb and monkey (3, 15, 19, 23, 25). Studies in the neonatal lamb have demonstrated the importance of hypothermia in initiating the neonatal TSH surge (22). Detailed observations of the pattern of circulating TSH concentrations in the fetus or of the regulation of TSH release have not been reported.

First we investigated circulating TSH concentrations in the chronically instrumented sheep fetus, lamb, and ewe, and present evidence of marked pulsatility of fetal TSH release. Second, we used an experimental preparation in which the ovine fetus can be cooled *in utero* (12, 13) to investigate the ontogenesis of hypothalamic control of TSH release.

METHODS

Animals. Pregnant Romney ewes of known gestational age mated with Suffolk rams were used in these studies. Term gestation in this breed is 145–150 days.

Operative procedures. In 33 fetuses between 86–132 days of gestation, polyvinyl catheters were implanted under maternal general anaesthesia into the fetal carotid artery and jugular vein to enable collection of blood samples *in utero.* A further 19 fetuses (102–132 days) had thermistors (sensitive to ±0.01° C) placed in either or both the fetal rectum and frontal intracranial region to record fetal temperature; in addition a thermistor was placed in the amniotic sac to record amniotic temperature. The maternal temperature was also monitored by a thermistor inserted into the tarsal vein and advanced to lie in the inferior vena cava. A cooling coil consisting of 5 m of McGaw V4520 blood warming coil was either placed "externally" around the fetal thorax to achieve a lowering of the fetal environment (amniotic) temperature or "internally" by inserting a length of coil (30–40 cm) into the fetal stomach. The fetal temperature was able to be manipulated by this latter method, without stimulation of the cutaneous thermoreceptors. Finally the catheters, thermistors, and afferent and efferent limits of the coil were exteriorized through the maternal flank (12, 13).

Following surgery the ewes were housed in metabolic cages with a fixed lighting period of 0700–1900 h and a constant temperature of 21° C and a humidity of 50%. They were given free access to hay and water supplemented by alfalfa and sheep nuts. The procedures were approved by the Animal Ethical Committee of the University of Auckland.

Experimental protocols. Random Studies. From 25 fetuses, 42 single arterial blood samples were obtained for basal TSH measurement not less than 96 h following surgery. Some fetuses were sampled more than once over a range of gestation but were not represented more than once in each group for analytical purposes.

Serial studies. Serial sampling was performed every 15–30 min for 3–4 h on 14 fetuses between 91–139 days of gestation not less than 96 h following surgery. Ten studies were performed on eight fetuses in the gestational range of 91–119 days and nine studies were carried out on seven fetuses between 120–139 days of gestation. All samples from an experiment were measured in a single assay. In order to demonstrate the nature of TSH release postnatally, studies in infant lambs and adult sheep were also performed. In five lambs (6–22 days of age) 10 studies were performed in which sampling was performed via a chronic indwelling venous catheter every 20 min for 3–4 h. In three adult nonpregnant ewes, studies were conducted over 4–5 h periods during which samples were obtained at 20-min intervals.

All catheters were opened at least 1 h prior to sampling in all studies. Fetal arterial samples (0.5 ml) were taken at zero time for measurement of pH and blood gas levels and analyses were restricted to fetuses with an arterial $p_{O_2} \geq 18$ torr and pH $\geq$ 7.34.

Cooling studies. All studies were performed at least 4 days postoperatively. The fetuses were cooled by means of circulating cold water (14–18° C) at a rapid rate (300–780 ml/min) through either the external or internal coil for 60 min to produce a decrease in temperature of 2–4° C (12, 13). Similar experiments were also carried out in which a much slower rate (6–38 ml/min) of cooling for 60 min was employed to cause a fall in fetal temperature of 0.6–1.0° C over 20 min to approximate a state of summit metabolism as defined by Alexander and Williams (1). Fetal and maternal temperatures were measured at 2-min intervals by means of a 5-channel microprocessor controlled amplifier transducer unit connected to the thermistors (12). Fetal arterial samples (1.5 ml) were taken 60 or 30 min, 15 and 1 min prior to cooling and 15, 30, and 60 min during the cooling. The samples were centrifuged and the plasma stored at −20° C.

Ovine TSH measurement. Plasma ovine TSH was measured by a heterologous radioimmunoassay using reagents provided by the NIADDK but with a modified assay procedure to enhance sensitivity. Bovine TSH (NIADDK-bTSH I-1) was iodinated by a chloramine T method and purified on a Sephadex G-100 column (0.9 × 50 cm) eluted with 0.01 M PBS buffer pH 7.6 (containing 0.02% sodium azide). Aliquots of ^{125}I-bTSH were stored at −20° C for no longer than a month. bTSH (NIADDK-I-1, AFP-2906B) was used for standards in the range of 1–100 pg/tube (quoted potency 30 U/mg). The incubation volume was 0.5 ml and the assay buffer was 0.01 M PBS, 0.1% bovine serum albumin, 0.33% EDTA pH 7.6. Samples were analyzed in aliquots of 0.2 ml and diluted if necessary by oTSH-free plasma obtained from an adult sheep treated with thyroxine. After preincubation for 48 h at 4° C with rabbit anti-oTSH serum (NIADDK-anti-oTSH-1) at a final dilution of 1:800,000, ^{125}I-bTSH (20,000 cpm) was added and the incubation was continued for 18–24 h at room temperature. Following this, 1 ml of precipitant (5% polyethylene glycol 6000, 1% sheep anti-rabbit γ globulin, 0.4% normal rabbit serum in 0.01 M PBS) was added. This was incubated for a further 15 min at room temperature, then centrifuged and the pellet counted. The minimal detectable dose of oTSH in the assay was 0.10 μU/ml (0.7 pg/tube). The half maximal displacement value was 1.3 μU/ml (9.1 pg/tube). Fetal and neonatal plasma diluted in parallel to the standard curve. The recovery of added TSH to TSH-free serum was linear over the concentration range of assay standards used. The within-assay coefficient of variation was 8% and the interassay coefficient of variation was 13.3%. Cross-reaction with highly purified preparations of oLH (Papkoff G3-222B) and oFSH (Papkoff G4-150C) was less than 1%. Ovine TSH concentrations are expressed in μU/ml to enable comparison to previous reports of fetal TSH concentrations.

Statistical analysis. We defined a pulse in TSH as an incremental change in plasma TSH concentrations greater than three times the estimated SD of the measured plasma TSH concentration of the preceding or following nadir and with a minimum amplitude of 1μU/ml. The SD was derived from the within assay coefficient of variation. Pulses in which the peak value occurred at the time of the initial or final sample were included. The pulse frequency was determined by dividing the number of pulses present by the total number of hours of the sampling period.

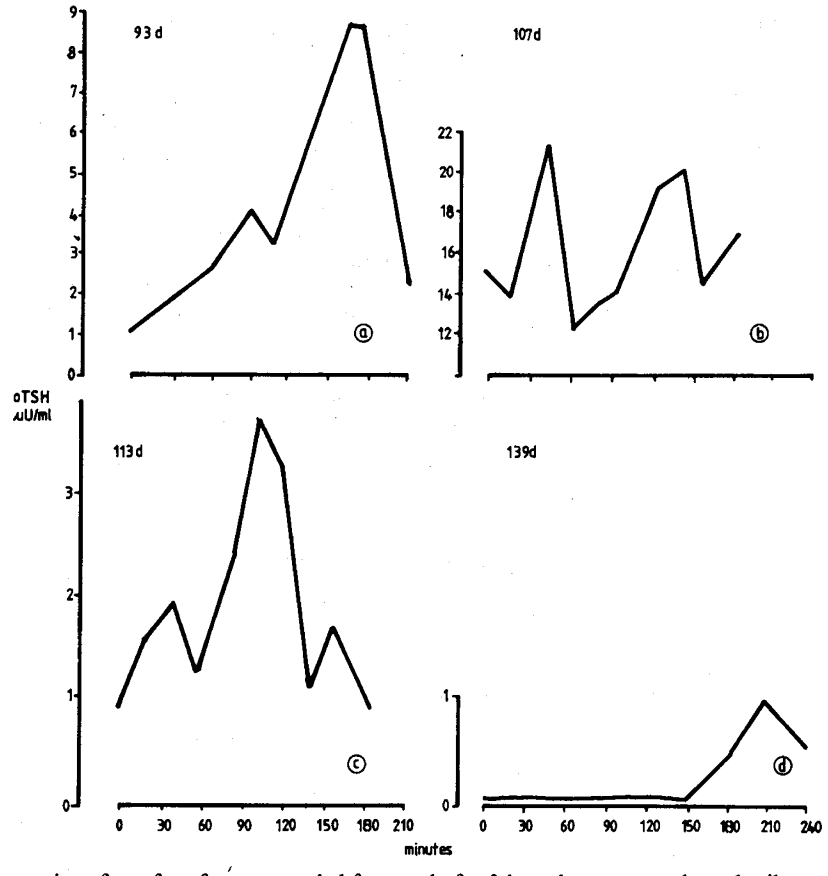

Fig. 1. Plasma TSH concentrations from four fetuses sampled frequently for 3 h to demonstrate the pulsatile nature of fetal TSH release. The gestational age of each fetus is shown.

Where no pulse was seen, the pulse frequency was expressed as <1.0 pulse/total number of hours for calculation purposes. The pulse amplitude was evaluated by the measurement of the differences between peak level within a pulse and the lowest value within the sampling period. Logarithmic transformation was applied to data from the cooling experiments before statistical evaluation. Except where otherwise stated comparisons were by paired or unpaired *t* test. The data are expressed as mean ± SD if not otherwise indicated.

RESULTS

Random hormone concentrations. The mean plasma TSH concentrations for each gestational age were: 86–110 days, 3.9 ± 5.5 μU/ml (range <0.1–16.0 μU/ml, $n = 13$); 111–130 days, 1.2 ± 1.1 μU/ml (range <0.1–4.3 μU/ml, $n = 16$); and 130–150 days, 0.4 ± 0.6 μU/ml (range <0.1–2.1 μU/ml, $n = 13$) which was less ($p < 0.05$) than either of the two younger groups. There was a broad range of random fetal TSH concentrations particularly in the younger fetuses.

Pulse studies. Serial sampling in fetuses demonstrated that there was significant variation in fetal TSH concentrations over the period of sampling suggestive of pulsatile release of TSH in the fetus. Examples of serial sampling demonstrating pulsatile release of TSH are shown in Figure 1. In eight of 10 studies performed on eight fetuses between 91–119 days gestation, pulses of TSH were observed. Between 120–139 days, five of nine studies on seven fetuses showed TSH pulses.

The range of amplitudes of all fetal TSH pulses observed between 91–139 days was 1.1–34.5 μU/ml (median 2.9 μU/ml) with a mean of 5.9 ± 8.1 μU/ml. For fetuses in the range of 91–119 days the mean amplitude was 7.4 ± 9.6 μU/ml and after 120 days the mean amplitude was 3.0 ± 1.4 μU/ml ($p < 0.1$) (Table 1).

Table 1. *Amplitude of TSH pulses in ovine fetus, lamb, and adult ewe*

	No. of fetuses	Total no. of studies	Pulse amplitude (μU/ml)*	p†
Fetuses	12	19	5.9 ± 8.1 (0.4–34.5)	
Lambs	5	10	2.1 ± 1.1 (0.2–3.8)	<0.005
Ewes	3	6	1.5 ± 0.4 (0.9–1.72)	<0.005

* Mean ± SD (range).
† Mann-Whitney test compared to fetuses.

In the 10 studies performed on five lambs (6–22 days) the pulse amplitude ranged from 1.0–4.1 μU/ml (median 1.6 μU/ml). The mean amplitude (2.1 ± 1.1 μU/ml) was significantly less than that of fetuses between 91–139 days (Mann Whitney, $p < 0.005$). In the six studies performed on three ewes only a few pulses of low amplitude were observed. The amplitude (range 1.0–7.7 μU/ml; mean, 1.45 ± 0.4 μU/ml) was not significantly different from that observed in postnatal lambs, but was less (Mann Whitney, $p < 0.05$) than that of the fetuses.

The lowest TSH value observed during each sampling period was considered as an index of basal TSH secretion. In the fetuses this value varied from 0.1–12.6 μU/ml, with a mean of 2.1 ± 3.8 μU/ml ($n = 19$). It was higher (Mann Whitney, $p < 0.001$) in the fetuses prior to 120 days gestation (3.4 ± 4.8 μU/ml, $n = 10$) than in older fetuses (0.42 ± 0.45 μU/ml, $n = 9$). The basal

Table 2. *Estimated pulse frequency in ovine fetus, lamb, and adult ewe*

	Total pulses/ total h observed	Pulse frequency pulses/h*
Fetuses	29/51	0.73 ± 0.30
Lambs	7/37†	0.33 ± 0.14
Ewes	3/27†	0.28 ± 0.11

* Mean ± SD.
† $p < 0.001$ by χ^2 analysis compared to all fetuses.

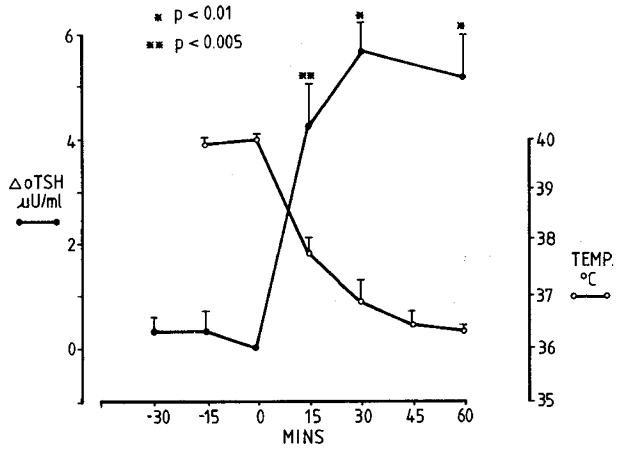

Fig. 2. The effect of fast cooling via an external coil on fetal plasma TSH concentrations (μU/ml ●) and fetal intracranial temperature (°C ○) in fetuses between 106–127 days of gestation. The TSH concentrations are expressed as the incremental change (Δ) from the immediate pre-cooling value. Cooling started at 0 min and continued for 60 min. * $p <$ 0.001. The mean ± SEs are shown.

TSH value for the lambs and ewes was <0.1 μU/ml in each study.

The pulse frequency data are summarized in Table 2. Twenty-nine pulses were observed during a total of 51 h of observation of the fetuses. The mean pulse frequency was 0.73 ± 0.30 pulses/h and ranged from <0.25–1.5 pulses/h. The pulse frequency was not different in fetuses less than 120 days of gestation from that in older fetuses.

There was a lower frequency of pulses observed during early postnatal and adult life. In the newborn lambs, the pulse frequency ranged from 0.25–0.67 pulses/h with a mean of 0.33 ± 0.14 pulses/h ($n = 10$). Seven pulses were demonstrated during 37 h, which was less than ($p < 0.001$) that measured in the fetuses. The ewes had a similar mean rate of pulses (0.28 ± 0.11 pulses/h) to postnatal lambs. Only three pulses were apparent during a total of 27 h of study which was of a lower frequency than that observed during fetal life ($p < 0.001$).

Cooling studies. Eleven external cooling experiments using a fast rate of water flow were performed on eight fetuses between 106–127 days of gestation. Prior to cooling the mean plasma TSH concentration was 1.5 ± 1.6 μU/ml. With cooling the plasma TSH concentration rose to a maximum of 7.4 ± 1.9 μU/ml ($p < 0.001$) after 30 min of cooling and was 6.7 ± 2.1 μU/ml ($p < 0.001$) at 60 min of cooling. The fetal intracranial temperature fell by 3.64 ± 0.50° C during the cooling period (Fig. 2).

Five fast internal cooling studies were performed on four fetuses (116–132 days of gestation). Plasma TSH concentrations rose with cooling in each experiment. Within 15 min of cooling the plasma TSH concentration rose from 1.4 ± 1.9 to 5.8 ± 7.9 μU/ml ($p < 0.02$) and was 5.9 ± 8.2 μU/ml ($p < 0.02$) after 1 h of cooling. The intracranial temperature fell by 1.94 ± 0.01° C with internal cooling.

There was a more variable response in the fetuses cooled at slower rates. Of the six fetuses cooled slowly by the external coil between 112–130 days of gestation, two of the six fetuses showed a TSH rise (increment 3.0, 3.6 μU/ml). Four fetuses (126–130 days) were slowly cooled via the stomach coil; three showed a rise in plasma TSH concentrations (increment 3.3, 3.5, 12.6 μU/ml).

DISCUSSION

In the present study we have demonstrated that in the ovine fetus, TSH is secreted in a pulsatile manner as early as 91 days of gestation. This pulsatility of fetal TSH secretion has not been reported previously. Studies in adult man, monkeys, and rats have demonstrated the existence of short-term variations (or pulsatile secretion) in plasma TSH (7, 18, 20, 29) albeit of relatively low amplitude. The distinct feature of the present observations is the markedly greater magnitude of this variability in the fetus compared to the lamb or adult ewe.

Previous studies in the ovine fetus have been limited to only single plasma samples for the measurement of TSH (27, 28) and cannot be directly compared with the present studies in which serial sampling at frequent intervals for several hours has been employed. Those previous observations either failed to demonstrate any maturational change in fetal TSH concentrations (27) or showed a trend to decrease near parturition (28). The present observations demonstrate the need for serial sampling to evaluate fetal TSH release—single estimations could be misleading given the magnitude and frequency of the pulsatility observed. We have recently found a marked pulsatility of fetal growth hormone secretion in the lamb of considerably greater magnitude than that observed after birth (8, 9). Gonadotropin secretion is also pulsatile in the fetal lamb (4). Studies in fetal endocrinology should therefore be designed so that the pulsatile nature of hormone release is taken into consideration.

The TSH pulses are not synchronous with growth hormone pulses (9) suggesting that the stimulus for pulsatile release of TSH is specific to the thyrotropic axis. Exogenous TRF has been demonstrated to stimulate fetal TSH release in the sheep (15, 25) and TRF is present in the fetal sheep hypothalamus by 60 days of gestation (D. M. Styne, P. D. Gluckman, P. L. Mueller, S. L. Kaplan, M. M. Grumbach, unpublished observations). Recently we have observed that the TSH pulse amplitude is significantly reduced by electrolytic lesions of the hypothalamus in the ovine fetus (8). Therefore it seems probable that TSH pulses reflect pulsatile hypothalamic TRF release.

We postulate that the exaggerated pulsatility of TSH secretion in the fetus is a consequence of less efficient negative feedback by iodothyronines in the fetus. This could be either a consequence of an immaturity of the negative feedback mechanism or due in part to the low concentrations of T_3 in the fetal circulation as a consequence of placental inner ring deiodinase activity directing thyroxine deiodination to reverse triiodothyronine (5, 21). In the sheep fetus, circulating T_3 levels are very low throughout gestation although they rise slightly in the last week before birth as a consequence of increasing glucocorticoid concentrations (2, 16). Available data support both an immature feedback mechanism and the low levels of circulating iodothyronines contributing to the distinct pattern of TSH release in the fetus.

In the postnatal lamb and monkey, TSH responsiveness to TRH is significantly lower than that seen in late gestation (3, 15). Further, the TSH response to TRH was not abolished by administration of T_3 to the sheep or monkey fetus in a dose effective in suppressing TSH release in the adult (15, 19). Other evidence for the delayed maturation of negative feedback is provided by clinical observations (6, 17). These studies demonstrate a pattern of increasing thyroid hormones in the face of decreasing TSH values between 30 wk gestation and 1–2 months of postnatal life. This suggests that feedback inhibition of pitui-

tary TSH production in man is developing but is not fully established until the postnatal period.

We also observed that basal plasma TSH concentrations in the fetal circulation decrease after 120 days. This decrease suggests that the change in the control of TSH secretion commences prior to birth in the fetal sheep presumably reflecting maturational changes in the hypothalamic-pituitary axis. The more marked change at birth associated with a significant decrease in pulse amplitude and frequency may reflect both a continuation of these maturational changes and the more marked postpartum increase for circulating triiodothyronine concentrations.

At birth there is a marked surge in plasma TSH concentrations associated with an increase in triiodothyronine secretion. The studies of Fisher and his colleagues (22) have demonstrated that this rise in TSH concentrations is consequent upon the fall in environmental temperature at birth whereas the rise in T_3 also depends on separation from the placenta so that the dominant effect of the inner ring deiodination of thyroxine to reverse triiodothyronine is removed (5, 21). These processes are believed to play a major role in the thermogenic adaptation of the neonate. Relative immaturity of the negative feedback loop may prevent excessive inhibition of neonatal TSH release by the increase in circulating triiodothyronine concentrations and this may be important in the continued secretion of thyroid hormones in the immediate neonatal period.

As hypothermia-induced TSH release in the neonate is mediated by hypothalamic mechanisms, we took advantage of our experimental approach for the *in utero* cooling of the fetus to evaluate the maturation of neuroendocrine mechanisms controlling fetal TSH release. Both a reduction in fetal core temperature alone (internal cooling) or associated with stimulation of fetal cutaneous thermoreceptors (external cooling) were powerful stimuli to fetal TSH release when the rate of cooling was rapid. Even when a more graduate change in fetal temperature was used to induce a state of summit metabolism (1), an increase in fetal TSH concentrations was observed in some fetuses.

Cold-induced TSH release was demonstrable by 106 days of gestation (0.7 gestation). Thus, the hypothalamic mechanisms to stimulate TSH release have differentiated by this age. Studies of anencephalic infants suggest that the hypothalamus may regulate TSH release in the human at birth (2, 14). In contrast, studies in the neonatal rat suggest that the hypothalamus does not exert an important influence on fetal TSH release (24). However, the neuroendocrine axis in the neonatal rat is more immature than in the late gestation fetal lamb or human infant (10).

These studies also demonstrate that the hypothalamic mechanisms by which hypothermia leads to appropriate neuroendocrine responses have differentiated by 106 days. This implies that at least certain aspects of hypothalamic thermoregulatory function are mature in midgestation. Our previous studies have also demonstrated that appropriate shivering and cardiovascular responses are differentiated by this age (11). Despite differentiation of these thermoregulatory mechanisms, the ovine fetus appears to have deficient thermogenesis *in utero* (13), presumably reflecting immaturity of heat generating systems rather than the regulatory components of the thermogenic axis.

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