Isoforms and half-life of FSH from sheep with different reproductive states

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Abstract

The glycoprotein hormone FSH comes in many different isoforms. In humans and rats the charges of the FSH isoforms vary with reproductive state and these affect the half-life of FSH in plasma. In this study we examined the charge heterogeneity of FSH in pituitary extracts from sheep with different reproductive states. Also the half-life of clearance of pituitary FSH from the different reproductive states was determined in mice. Pituitaries were collected from: anoestrous, luteal phase, follicular phase, early-pregnant and late-pregnant ewes, ewe lambs, ram lambs, rams during the breeding and non-breeding seasons and wethers (5 per group). After extraction, FSH isoforms were fractionated by HPLC anion exchange chromatography. The volume at which half of the FSH had eluted from the ion exchange column was determined (HP_{50}). It was found that FSH isoforms from ewes ($HP_{50} = 96.7 \pm 1.3$ ml (S.E.M.)) eluted later ($P < 0.01$) than those from rams ($HP_{50} = 82.3 \pm 1.3$ ml) indicating that FSH isoforms in the ewes were more acidic than those from rams. There was a seasonal difference in ewes, with ewes in anoestrus ($HP_{50} = 101.6 \pm 2.6$ ml) having more-acidic ($P < 0.01$) FSH isoforms than the ewes during the oestrous cycle

($HP_{50} = 95.3 \pm 0.7$ ml). There was an effect of age, with the FSH isoforms from cycling ewes ($HP_{50} = 95.3 \pm 0.7$ ml) being more acidic ($P < 0.01$) than those from ewe lambs ($HP_{50} = 88.3 \pm 1.9$ ml). There was an effect of pregnancy, with late-pregnant ewes ($HP_{50} = 107.3 \pm 1.6$ ml) having more-acidic FSH isoforms ($P < 0.05$) than those from anoestrous ewes ($HP_{50} = 101.6 \pm 2.6$ ml) and there was an effect of castration with the breeding season rams ($HP_{50} = 80.7 \pm 1.4$ ml) having more-acidic ($P < 0.05$) FSH isoforms than wethers ($HP_{50} = 74.0 \pm 0.5$ ml). The half-life of pituitary FSH from animals in the different reproductive states was found to be negatively correlated with HP_{50} ($r^2 = 0.56$, $P < 0.01$). The FSH isoforms from wethers were the least acidic and had the longest half-lives. Collectively, these findings show that in sheep, age, sex and reproductive state are all factors which influence the forms of FSH that are extracted from the pituitary gland. Moreover, these results demonstrate that FSH from sheep with the most-acidic FSH isoforms have the shortest half-life in plasma.

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Introduction

The pituitary hormone follicle-stimulating hormone (FSH) is composed of two polypeptide subunits and four carbohydrate side chains. FSH, like other glycoprotein hormones, exists as a large number of isoforms. The heterogeneity which generates these isoforms originates from the variation in the oligosaccharide side chains. These oligosaccharides often terminate in electrically charged sugars, such as sialic acid or sulphated N-acetyl galactosamine, which influence the properties of FSH (Chappel *et al.* 1983a, Cooke *et al.* 1996, Stanton *et al.* 1996). Because the sugars are charged, the isoforms can be separated using techniques such as electrophoresis (Wide 1985), isoelectric focusing (Chappel *et al.* 1982), ion exchange chromatography (Phillips *et al.* 1995) and chromatofocusing (Blum & Gupta 1985). These techniques

have shown that the types of pituitary FSH isoforms that are produced are affected by reproductive status (Wide 1989, Beitins & Padmanabhan 1991, Ulloa-Aguirre *et al.* 1995). Studies in humans (Wide 1986) and rats (Blum & Gupta 1985) have shown that male FSH isoforms are more acidic and have longer half-lives than those from females of a reproductively active age. Also, in humans and rats when there is gonadal inadequacy such as before puberty, after menopause and after castration, FSH isoforms are more acidic and have longer half-lives than in other reproductive states (Cameron & Chappel 1985, Wide 1989). It is not known whether these changes in FSH isoforms occur in sheep. From the limited number of studies it appears that the relationship between the reproductive state of the sheep, the acidity of the isoforms and the half-life of the isoforms may be different from that in humans and rats (Blum & Gupta 1985, Wide 1986, Phillips *et al.* 1995,

Cooke *et al.* 1996). Currently, commercial preparations of ovine pituitary FSH (Ovagen; ICP Ltd, Auckland, New Zealand) are used for superovulating many different species including sheep, cattle and deer (McNatty *et al.* 1989, Henderson *et al.* 1990, Smith *et al.* 1993). However, little is known about the effect of age, sex or reproductive state on the properties of ovine FSH.

The aim of this study was to determine whether there is an effect of age, sex or reproductive state on the charge and the half-life of pituitary FSH and to examine if the average charge of sheep FSH isoforms is correlated with the circulatory half-life of FSH.

Materials and Methods

Ethics

All experiments were performed in accordance with the 1987 animal protection (codes of ethical conduct) regulations of New Zealand and had been approved by the animal ethics committee of the Wallaceville Animal Research Centre, Upper Hutt.

Pituitaries

Pituitary glands were collected from: luteal phase ewes at day 10 of the oestrous cycle; follicular phase ewes 36 h after a prostaglandin F₂ alpha (Estrumate; Pitman Moore, Upper Hutt, New Zealand) injection given at day 10 of the oestrous cycle; ewe lambs and ram lambs at 5 months of age; ewes at days 24 and 30 of pregnancy (early-pregnant); wethers at 2 years of age; and rams during the breeding season. Pituitaries from the above sheep were collected in May, which is about the middle of the breeding season in New Zealand. Pituitary glands were also collected from ewes between days 124 and 135 of pregnancy (late-pregnant) in August. Pituitaries from ewes in anoestrus and sexually inactive rams were collected in October, which is the middle of the non-breeding season in New Zealand.

Pituitary glands were collected from the sheep within 10 min of death, snap frozen in liquid nitrogen and stored at -70°C until extraction. Individual pituitary glands were extracted in 6 ml 5 mM phosphate buffer (pH 7.5) containing 0.1% BSA (Immuno-Chemical Products Ltd, Auckland, New Zealand) and 1 mM phenylmethylsulphonyl fluoride (Sigma Chemical Co., St Louis, MO, USA). After centrifugation of the homogenate at 4°C for 30 min at 30 000 *g* the precipitate was re-extracted as above. The supernatants were combined and then frozen and stored at -20°C (Robertson *et al.* 1982).

FSH RIA

The ovine FSH RIA used the United States Department of Agriculture USDA-oFSH-19-SIAFP-I-2 preparation

for the iodination reagent, USDA-oFSH-19-SIAFP-RP2 as the standard and the United States National Institute of Diabetes, Digestive and Kidney Diseases NIDDK-anti-oFSH-1 as the antiserum. The RIA was carried out as previously described (McNatty *et al.* 1987) with the exception that the iodinated FSH was purified by ion exchange chromatography (Moore *et al.* 1997). The mean intra- and interassay coefficients of variation were 8.8 and 6.6% respectively. The minimum detectable concentration of FSH was 0.2 ng/ml as defined by the concentration $2 \times \text{s.d.}$ from the 0 standard. The ED₈₀ value averaged 0.39 ng/ml and the ED₅₀ 1.29 ng/ml.

Half-life of FSH

The circulating half-life of the various FSH preparations was measured by injecting the sheep pituitary extracts i.v. into mice and measuring the rate of disappearance from the circulation as described by Phillips *et al.* (1995). Briefly, mice (three to five per time point) were injected with between 0.5 and 2 µg of immunoreactive pituitary FSH and were bled 5, 15, 30, 60 or 120 min later. Because ram and ram lamb pituitaries contained low amounts of FSH, two pituitary extracts were combined for each of these half-life determinations ($n=3$). Individual pituitary extracts were used for all the other half-life determinations. Mouse FSH was not crossreactive in the ovine FSH RIA.

High performance liquid chromatography (HPLC) anion exchange chromatography

The HPLC anion exchange chromatography was performed as described by Phillips *et al.* (1995). Briefly, 12% of each pituitary extract was filtered through a 0.45 µm Sartorius Minsart filter (Gottingen, Germany) and loaded onto an MA7Q anion exchange column (100 × 19 mm; Biorad, Richmond, CA, USA) connected to an HPLC (Hewlett Packard 1050, Waldbronn, Germany). The column was eluted at 4 ml/min with 20 mM Tris-HCl (pH 8.6) for 2 min. This was followed by a linear gradient from 0 to 400 mM KCl in 20 mM Tris-HCl (pH 8.6) over 50 min. Finally, the concentration of KCl was increased to 2 M for 4 min. Three-millilitre fractions were collected in test tubes containing 100 µl 10% (w/v) BSA in 20 mM Tris-HCl. The fractions were stored at -20°C until they were assayed. Prior to RIA the buffer in each fraction was exchanged for 50 mM Tris-HCl (pH 7.4) containing 0.3% BSA and 10 mM MgCl₂ by running 2 ml from each fraction through a PD10 column (Pharmacia, Uppsala, Sweden). The average recovery of FSH from the anion exchange column was $70.1 \pm 2.1\%$ (s.e.m.).

Comparison of chromatofocusing and HPLC anion exchange chromatography

A sheep pituitary extract was run on an HPLC anion exchange column and then the fractions which eluted at

Table 1 Mean $\pm$ S.E.M. pituitary weights, FSH concentrations and FSH contents from sheep of different reproductive states ($n=5$ per group)

	Pituitary weight (g)	Pituitary FSH concentration ($\mu\text{g/g}$)	Pituitary FSH content ($\mu\text{g/pit}$)	Half-life (min)
Follicular phase ewes	$0.89 \pm 0.09^{\text{de}}$	$26.1 \pm 5.0^{\text{a}}$	$21.6 \pm 3.0^{\text{a}}$	$8.8 \pm 0.9^{\text{a}}$
Luteal phase ewes	$0.91 \pm 0.09^{\text{de}}$	$21.1 \pm 3.1^{\text{a}}$	$19.2 \pm 2.9^{\text{a}}$	$9.6 \pm 0.4^{\text{a}}$
Early-pregnant ewes	$0.62 \pm 0.04^{\text{abcd}}$	$66.6 \pm 7.7^{\text{b}}$	$41.9 \pm 7.1^{\text{a}}$	$10.2 \pm 1.1^{\text{a}}$
Late-pregnant ewes	$0.66 \pm 0.05^{\text{bcde}}$	$24.0 \pm 4.9^{\text{a}}$	$16.4 \pm 4.1^{\text{ab}}$	$8.3 \pm 0.8^{\text{a}}$
Anoestrous ewes	$1.02 \pm 0.15^{\text{e}}$	$29.3 \pm 4.3^{\text{a}}$	$27.9 \pm 2.3^{\text{a}}$	$7.2 \pm 1.5^{\text{a}}$
Ewe lambs	$0.25 \pm 0.02^{\text{a}}$	$96.8 \pm 9.8^{\text{b}}$	$23.1 \pm 0.9^{\text{a}}$	$11.0 \pm 1.0^{\text{a}}$
Ram lambs	$0.39 \pm 0.11^{\text{abc}}$	$17.4 \pm 5.4^{\text{a}}$	$5.3 \pm 1.1^{\text{b}}$	$9.0 \pm 1.3^{\text{a}}$
Breeding season rams	$0.68 \pm 0.06^{\text{cde}}$	$9.8 \pm 1.6^{\text{a}}$	$6.5 \pm 1.1^{\text{b}}$	$11.5 \pm 1.7^{\text{a}}$
Non-breeding season rams	$0.73 \pm 0.09^{\text{cde}}$	$7.6 \pm 1.8^{\text{a}}$	$5.7 \pm 1.7^{\text{b}}$	$12.3 \pm 2.6^{\text{ab}}$
Wethers	$0.28 \pm 0.05^{\text{ab}}$	$80.1 \pm 3.8^{\text{b}}$	$22.4 \pm 2.5^{\text{a}}$	$18.2 \pm 2.0^{\text{b}}$

^{abcde}Within a column means with different superscripts are significantly different ($P<0.05$).

75, 90, 105 and 120 ml were loaded on to PD10 columns to change the buffer to 25 mM imidazole (pH 7.4). The fractions were then individually run on a chromatofocusing column (25×1 cm, Pharmacia) attached to an FPLC apparatus (Pharmacia). The chromatofocusing column was equilibrated with 25 mM imidazole (pH 7.4) and after loading was eluted with 12.5% Polybuffer 74 (Pharmacia) adjusted to pH 4. The eluting fractions were assayed for FSH in the RIA and the average pH at which each fraction eluted was calculated.

Statistical analyses

After assaying the fractions from the HPLC anion exchange column for FSH, the average charge of pituitary FSH was determined by calculating the volume at which 50% of the FSH had eluted (HP_{50}). The results were analysed by ANOVA and the following contrasts were made: ewes during the luteal phase with ewes during the follicular phase; ewes during anoestrus with ewes during the oestrous cycle; ewes in the breeding season with ewes in early pregnancy; ewes in anoestrus with ewes in late pregnancy; ewes in the breeding season with ewe lambs; ewes in the breeding season with rams in the breeding season; ewes in anoestrus with rams from the non-breeding season; ewe lambs with ram lambs; rams in the breeding season with ram lambs; rams in the breeding season with rams from the non-breeding season; and wethers with rams from the breeding season.

The half-lives of ovine FSH were determined by fitting the decay rate to a single exponential component curve using the Sigma Plot (Jandel, San Rafael, CA, USA) graphical package.

Comparison of pituitary weights, FSH half-lives, FSH pituitary concentrations and FSH contents was done by ANOVA with Tukey's *post hoc* test.

The relationship between the HP_{50} and half-life was analysed using linear regression.

Results

Pituitary FSH concentration and content

Sheep pituitary weights, FSH concentrations and FSH contents are shown in Table 1. Ewe lambs, ewes during early pregnancy and wethers had pituitary FSH concentrations which were higher than in the other groups ($P<0.01$). The three ram groups had pituitary FSH contents which were lower than ($P<0.05$) those in the wethers and in all groups of ewes except for late-pregnancy. The non-pregnant ewes had heavier ($P<0.05$) pituitaries than the wethers and the ewe and ram lambs.

Comparison of chromatofocusing and HPLC anion exchange chromatography

The FSH from an HPLC anion exchange column that eluted at 75, 90, 105 and 120 ml eluted at average pHs of 5.30, 4.93, 4.54 and 4.50 respectively when run on a chromatofocusing column.

HPLC ion exchange reproducibility

A quality control pituitary extract, which was run approximately every tenth HPLC run, had an HP_{50} of 73.7 ± 0.7 ml (S.E.M., $n=6$). Rerunning fraction 24 from an HPLC run resulted in a defined peak of FSH activity centred on fraction 24 (Fig. 1).

HPLC ion exchange

Mean FSH profiles for the ewes during the follicular and luteal phases and the rams during the breeding season following HPLC ion exchange are shown in Fig. 2. No difference between the mean profiles of the follicular phase and the luteal phase ewes was observed. However, both groups of ewes contained FSH isoforms which eluted later than the isoforms from rams in the breeding season

Figure 1 FSH activity after HPLC anion exchange chromatography of a pituitary extract from a ewe during anoestrus (●) and the rechromatography of fraction 24 (○) on the same column.

Figure 2 Mean $\pm$ S.E.M. FSH activity after HPLC anion exchange of pituitary extracts from rams during the breeding season (□; $n=5$), ewes during the follicular phase (○; $n=5$) and ewes during the luteal phase (●; $n=5$). R, F and L are the mean HP_{50} values for the rams during the breeding season, ewes during the follicular phase and ewes during the luteal phase respectively.

(Fig. 2). The HP_{50} s for all the groups are shown in Fig. 3. There was no difference in the mean FSH HP_{50} between ewes collected during the luteal (95.2 ± 0.7 ml) and the follicular (95.4 ± 1.4 ml) phases (Fig. 2). There was a significant effect of season in ewes with the mean HP_{50} being higher during the non-breeding season (101.6 ± 2.6 ml) than during the breeding season (95.3 ± 0.7 ml) ($P<0.01$). However, there was no effect of season in rams, with the mean HP_{50} during the non-breeding season (84.5 ± 2.5 ml) not significantly different from that during the breeding season (80.7 ± 1.4 ml). Comparison of HP_{50} s from 5-month-old ewe lambs (88.3 ± 1.9 ml) with ewes during the breeding season (95.3 ± 0.7 ml) showed a significant difference ($P<0.01$). There was no difference between ram lambs (81.6 ± 2.7 ml) and rams during the breeding season (80.7 ± 1.4 ml) or between early-pregnant ewes (92.5 ± 2.1 ml) and ewes during the oestrous cycle (95.3 ± 0.7 ml). However, the mean HP_{50} from ewes in late pregnancy (107.3 ± 1.6 ml) was

Figure 3 Mean $HP_{50} \pm$ S.E.M. of pituitary FSH from sheep with the following reproductive states: ewes during the follicular phase (FP), ewes during the luteal phase (LP), days 24–30 of pregnancy (EP), days 120–135 of pregnancy (LPG), ewes during anoestrus (AE), ewe lambs (EL), ram lambs (RL), rams during the breeding season (BSR), rams during the non-breeding season (NBR) and wethers (W) ($n=5$ per group). The following significant differences in HP_{50} values were observed: ewes during the follicular and luteal phases were lower than ewes during anoestrus ($P<0.01$); ewes during the follicular and luteal phases were higher than ewe lambs ($P<0.01$); ewes during late pregnancy were higher than ewes during anoestrus ($P<0.05$); ewes during the follicular and luteal phases were higher than the rams during the breeding season ($P<0.01$); ewes during anoestrus were higher than rams during the non-breeding season ($P<0.01$); ewe lambs were higher than ram lambs ($P<0.01$) and rams from the breeding season were higher than wethers ($P<0.05$).

significantly higher than for ewes during anoestrus (101.6 ± 2.6 ml) ($P<0.05$). There was a significant effect of sex with the ram lambs (81.6 ± 2.7 ml), rams during the breeding (80.7 ± 1.4 ml) and non-breeding seasons (84.5 ± 2.5 ml) having significantly lower mean HP_{50} s than ewe lambs (88.3 ± 1.9 ml) ($P<0.05$), ewes during the breeding (95.3 ± 0.7 ml) ($P<0.01$) (Fig. 2) and non-breeding seasons (101.6 ± 2.6 ml) ($P<0.01$). The mean HP_{50} of the rams during the breeding season (80.7 ± 1.4 ml) was higher than those of the wethers (74.0 ± 0.5 ml) ($P<0.05$), indicating an effect of castration.

Half-life and correlation of HP_{50} with half-life

FSH from wethers had a longer ($P<0.05$) mean half-life in mice than FSH from all the other groups except for extracts from rams during the non-breeding season (Table 1).

The half-life of FSH was negatively correlated with HP_{50} ($r^2=0.56$, $P<0.01$) (Fig. 4). The wethers had the longest half-lives and the smallest HP_{50} values.

Discussion

This study has shown that in sheep that there are significant differences in pituitary FSH isoforms between

Figure 4 The relationship between the half-life in mice and the HP_{50} of pituitary extracts of FSH. $r^2=0.56$, $P<0.01$.

different reproductive states. The first FSH isoforms to elute from the HPLC anion exchange column are the least acidic and the least negatively charged, whereas the last to elute are the most acidic and most negatively charged isoforms. The later-eluting isoforms are likely to have the highest concentrations of sialic acid or sulphated sugar residues. The HPLC anion exchange method separates FSH isoforms in a similar manner to agarose zone electrophoresis (Wide 1985), which is also usually performed at pH 8.6. The mean volume or HP_{50} at which FSH isoforms elute from the HPLC column is directly related to the median volume or albumin mobility unit (AMU) when FSH isoforms elute from a zone electrophoresis column ($HP_{50}=320 \text{ AMU} - 54$, $r=0.80$) (Phillips *et al.* 1995). The ion exchange method also correlates with the chromatofocusing method with the fractions eluting first on the HPLC ion exchange column having the highest isoelectric points.

In our study on sheep FSH isoforms, we found that females have more-acidic FSH isoforms than males. This is the opposite to what has been reported for prepubertal children (Wide 1989), humans of reproductive age (Wide 1989) and rats (Robertson *et al.* 1982, Blum *et al.* 1985).

Ewes have more-acidic pituitary FSH isoforms than ewe lambs. Wide (1989) found no difference between the median charge of pituitary FSH isoforms from girls and women of reproductive age. Results in adult and prepubertal female rats have been inconsistent; Blum & Gupta (1980) found adult female rats had more-acidic pituitary isoforms than prepubertal female rats while Chappel *et al.* (1983b) observed the opposite.

With respect to age of male sheep, we found no difference in the charge between lambs and adults. In contrast, Wide (1989) found that men had more-acidic FSH isoforms than boys, whereas in adult male rats FSH

isoforms were less acidic than those in prepubertal animals (Chappel & Ramaley 1985).

No changes were found in pituitary FSH isoforms between the luteal phase and the follicular phase ewes, a finding similar to that observed in cattle (Kojima *et al.* 1995). Ovine pituitary luteinizing hormone (LH) isoforms have also been found to be similar during the oestrous cycle (Zalesky *et al.* 1992). In rats there is an increase in the least-acidic pituitary FSH isoforms when there is an increase in gonadal activity prior to oestrus (Ulloa-Aguirre *et al.* 1988).

When there is gonadal inadequacy in humans, such as after menopause and prepuberty, pituitary FSH isoforms are more acidic (Wide 1989). Apart from ewes during anoestrus, which have more-acidic isoforms than ewes during the oestrous cycle, sheep do not appear to conform to the pattern observed in humans during gonadal inadequacy because ewe lambs, ovariectomized ewes (Phillips *et al.* 1995) and wethers have less-acidic pituitary isoforms than their gonadally active counterparts. Sheep are similar to rats (Ulloa-Aguirre *et al.* 1986) and cows (Stumpf *et al.* 1992) where gonadectomy also increases the amount of less-acidic pituitary FSH isoforms. Similar to what happens with ovine FSH, pituitary ovine LH becomes more acidic during anoestrus (Zalesky *et al.* 1992) and rams have more-acidic LH isoforms than wethers (Keel *et al.* 1987, Zalesky *et al.* 1993).

The more-acidic pituitary FSH isoforms in rams compared with adult wethers is probably due to an effect of androgens because the isoform pattern of wether lambs can be returned to that of ram lambs when wether lambs are treated with dihydrotestosterone (Keel & Schanbacher 1987). Testosterone increases the amount of the least-acidic serum FSH isoforms in men (Dahl & Stone 1992) and the least-acidic FSH isoforms in male rat anterior pituitary cells (Kennedy & Chappel 1985). Treatment of male rats with a non-steroidal anti-androgen causes a shift to less-acidic pituitary FSH isoforms (Simoni *et al.* 1992). However, the shift caused by anti-androgen is not as great as that caused by castration. Because inhibin is greatly reduced by castration but not by androgen blockade this result suggests that inhibin might also be regulating the types of FSH isoforms that are produced (Simoni *et al.* 1992). Steroid-free follicular fluid, which contains inhibin, causes a decrease in the least-acidic pituitary FSH isoforms in ovariectomized hamsters (Galle *et al.* 1983).

Oestradiol increases the amount of the least-acidic serum FSH isoforms in women (Padmanabhan *et al.* 1988, Wide & Naessen 1994) and men (Wide 1982, Matikainen *et al.* 1994). Treatment of wethers with oestradiol has the opposite effect and increases the amount of acidic pituitary isoforms (Keel & Schanbacher 1987). Previously we have shown that ovariectomy decreases the acidity of the isoforms in sheep (Phillips *et al.* 1995). Consistent with oestradiol increasing the amount of acidic isoforms, in this study we found that FSH isoforms were more acidic in the

higher oestrogenic states of late pregnancy compared with anoestrous ewes and in ewes compared with rams. However, when the ewes were exposed to high concentrations of oestrogen during the follicular phase compared with the luteal phase there was no effect on the FSH isoform profile. Also when anoestrous ewes were compared with cycling ewes it was the anoestrous ewes, with the lowest oestrogen levels, which had the most-acidic FSH isoforms. Therefore it seems that the FSH isoform distribution pattern in sheep is not controlled solely by oestrogen.

In humans, reproductive states which have a high gonadotrophin-releasing hormone (GnRH) pulse frequency are associated with increases in the amount of the least-acidic FSH isoforms in serum (Wide & Albertsson-Wikland 1990, Phillips & Wide 1994, Wide *et al.* 1996). Consistent with this pattern, pituitary FSH isoforms from ovariectomized ewes are less acidic than those from ewes during the oestrous cycle (Phillips *et al.* 1995). Also FSH isoforms from wethers are less acidic than those from rams, and ewe lambs are less acidic than those from adult ewes. However, in other comparisons where the secretion of GnRH is different, such as between luteal phase and follicular phase ewes, between ram lambs and adult rams or between rams during the non-breeding season and breeding season, no differences in the FSH isoform distributions were observed. When anoestrous ewes were compared with cycling ewes it was the anoestrous ewes with the lowest GnRH pulse frequency which had the most-acidic FSH isoforms. Overall, there does not appear to be a consistent effect of GnRH on the ovine FSH isoform distribution. In nutritionally restricted ovariectomized lambs, Hassing *et al.* (1993) found no evidence of an effect of GnRH on the pituitary, secreted or serum FSH isoform distributions. However, in intact ewe lambs pulsatile GnRH increased the amount of the least-acidic serum FSH isoforms (Padmanabhan *et al.* 1992). This suggests that in the absence of gonadal hormones GnRH is unable to alter the FSH isoform pattern.

In this study we found that in sheep the most-acidic isoforms have the shortest half-lives. Consistent with this result is that FSH from ovariectomized sheep is less acidic (Phillips *et al.* 1995) and has a longer half-life than FSH from intact ewes (Robertson *et al.* 1991). In sheep there are more of the less-acidic FSH isoforms in serum than in the pituitary (Padmanabhan *et al.* 1992) despite the pituitary secreting a similar profile of FSH isoforms to that found in pituitary extracts (Hassing *et al.* 1993). A possible explanation for this result is that the least-acidic isoforms are more abundant in serum than in the pituitary because the least-acidic isoforms have longer half-lives in serum than the acidic isoforms. The observation that sheep with the least-acidic FSH isoforms have the longest half-lives is the opposite to what happens in humans (Wide 1986) and rats (Blum & Gupta 1985) where the most-acidic FSH isoforms have the longest half-lives. The half-life of human FSH isoforms is correlated with sialic acid content (Morell

et al. 1971). Sheep FSH is not as rich in sialic acid as human FSH but it contains more sulphated residues (Green & Baenziger 1988), which also make FSH isoforms more acidic. These acidic sulphated FSH residues may cause a decrease in the half-life of ovine FSH (Fiete *et al.* 1991, Baenziger *et al.* 1992). However, it is worth noting that the least-basic forms of ovine LH, which is also highly sulphated, have the longest half-lives (Nakamura *et al.* 1993) and desulphation of bovine LH causes a decrease in its half-life (Baenziger *et al.* 1992). Nevertheless, a desulphated glycoprotein may have different properties to the native and less-acidic FSH isoforms found in the pituitary, and moreover ovine FSH may not behave in the same way as bovine LH.

Lower pituitary levels of FSH in rams compared with ewes has also been observed by Robertson *et al.* (1984) and is consistent with low plasma levels of FSH in rams (Bremner *et al.* 1980). In humans, males also have lower pituitary levels of FSH than females (Wide 1989). We think that this lower level of FSH in males than females may be due to negative feedback effects of testicular inhibin and androgens because we found that wethers have higher concentrations of pituitary FSH than rams.

In summary, the average charge of sheep FSH isoforms that are present in different reproductive states are often the opposite to what are present in humans. In humans and in sheep, gonadectomy causes the production of FSH isoforms with long half-lives. But whereas in humans the long half-life FSH isoforms are the most acidic, in sheep the long half-life FSH isoforms are the least acidic ones.

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