Individual variations in FSH release after a GnRH challenge and relationship with semen output in young bulls

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Summary. Blood samples collected from 54 Montbéliarde young post-pubertal bulls were assayed for FSH. In the first trial, 0.25 mg GnRH was given to each of 25 bulls at 12 months of age. In a second group of 29 bulls, each received 20 mg dexamethasone followed by 0.25 mg GnRH 5 h later. Based on measurements of semen output, the bulls were classified into three categories: good, medium and poor semen producers. The total FSH response was significantly different among individuals ($P < 0.05$) and repeatable ($r = 0.45$) only after the combined treatment. The mean total responses did not differ significantly between the 3 categories of semen producers, but individual FSH responses were significantly and negatively correlated to quantitative and qualitative semen production characteristics ($r = 0.4-0.7$).

It was concluded that measurement of FSH after a combined dexamethasone–GnRH challenge permits the demonstration of a significant relationship between FSH release and semen production in individual bulls.

Introduction

There is now considerable evidence for animals of many species that a GnRH challenge induces a significant release of both FSH and LH (Pelletier, 1976; Schally, 1978). However, because of the difficulties with appropriate and homologous systems of FSH measurement (Cheng, 1978; Bolt & Rollins, 1983; Butler, Katz, Arriolo, Milvae & Foote, 1983), this has not been so widely investigated for bulls (Schanbacher & Echterkamp, 1978; Bass, McNeilly & Moreton, 1979). It has been previously shown that the LH response to GnRH or to a dexamethasone–GnRH combined treatment was able to discriminate significantly between individual bulls based on the magnitude of this release. However, this response did not seem to be related to semen output even with careful measurement of the semen characteristics (Abdel Malak & Thibier, 1982).

Because FSH has a major direct action on germ cell production (Amann, 1983), it seemed of interest to study the individual FSH responses to both the GnRH and dexamethasone–GnRH treatments and to study their relationship to the responses that have been previously reported (Abdel Malak & Thibier, 1982). An alteration of the FSH pattern was detected in bulls only in an azoospermic individual with Klinefelter's syndrome (Abdel Malak, Cribiu & Thibier, 1984), in contrast to other species such as man (Franchimont *et al.*, 1972). Spontaneous FSH profiles were not affected in other types of azoospermic bulls, such as those with the Sertoli cell-only syndrome or excretory–secretory type, when compared to contemporary normospermic bulls (Abdel Malak & Thibier, 1984).

Using the same blood samples from the bulls previously referred to (Abdel Malak & Thibier, 1982), this study was aimed at investigating (1) the FSH patterns and their repeatability within individuals, after such challenges, and (2) their possible relationship to semen output characteristics.

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Materials and Methods

Experimental animals and semen analysis. The animals and method of analysis have been previously described (Abdel Malak & Thibier, 1982). In brief, 54 Montbéliarde bulls kept in the same performance test station (01250 Ceyzériat) were involved. They were trained to ejaculate into an artificial vagina from the age of 43 weeks and then two successive ejaculates were collected on a weekly basis for 15 consecutive weeks (51–65 weeks of age). Sperm output of each individual was carefully investigated immediately after semen collection as previously described (Thibier & Colchen-Bourlaud, 1972). The main criteria taken into account were semen volume, sperm motility and sperm density. The total number of spermatozoa was calculated by multiplying semen volume by sperm density. Semen smears were made and stained with nigrosin-eosin to determine the percentage of spermatozoa with abnormal morphology (total, head, tail mid-piece, decapitated spermatozoa) only for the 2nd ejaculates every other week because of its high repeatability (Colchen-Bourlaud & Thibier, 1973). All evaluations were performed by the same person throughout the course of the study.

From these data, bulls were classified as having good, medium or poor sperm output.

Hormonal challenges and blood sampling. As already reported, the first 25 bulls were given a single i.m. injection of 0.25 mg GnRH (Intervet, 49005 Angers, France) on two occasions 1 month apart (at 12 and 13 months of age). The other 29 bulls received an i.m. injection of dexamethasone (20 mg; Dexadresone; Intervet, France) and 5 h later were challenged with an i.m. injection of GnRH (0.25 mg). This combined treatment was given at 12 and 13 months of age. In the first group, 9 bulls were classified as good semen producers, 9 medium and 5 as poor; in the other group, the numbers were respectively 12, 10 and 7.

The blood sampling regimen for the single GnRH challenge was as follows: samples were taken every 15 min before and for the first 150 min after the GnRH injection and then every 60 min during the subsequent 4 h (15 samples altogether per individual). For the combined injections of dexamethasone-GnRH, blood sample collection began just before the dexamethasone injection and samples were taken at 1-h intervals for 5 h. GnRH was then injected and the sampling regimen was similar to that described above.

The blood samples were obtained by venepuncture of the jugular vein and collected into heparinized vacutainer tubes. The animals were accustomed to human handling and the samples could be taken with minimal restraint. Immediately after collection the blood samples were centrifuged at 3000 g for 10 min and plasma was separated and stored at -20°C until required for FSH assay.

Hormonal assays. The plasma concentrations of FSH were determined by using the double-antibody precipitating method of radioimmunoassay as previously described (Abdel Malak, 1983; Abdel Malak *et al.*, 1984). The system was homologous and adapted from that of Cheng (1978) by Desoutter (1981) in our laboratory. The purified bovine FSH preparation used as the standard was b-FSH C.K. (164 $\times$ NIH-FSH-S1). The antiserum (from Dr Cheng) was used at a 1/60 000 final dilution in phosphate-buffer solution (0.05 M; pH 7.4) to which was added 4 g human albumin per litre. Cross-reactivities were negligible with bovine LH ($< 0.1\%$) and bovine TSH ($< 3\%$). This result was in agreement with that reported by Cheng (1978). The detection limit was 0.1 ng/ml for b-FSH C.K. Intra- and inter-assay coefficients of variation were, respectively, 8.3% (mean = 1.92 ng/ml; 10 replicates) and 10.2% (mean = 2.72 ng/ml; 12 replicates).

Statistical analyses. The results are given in terms of mean $\pm$ s.e.m. and comparisons were statistically evaluated by Student's *t* test. The repeatability was estimated by calculation of the intra-class coefficient of correlation according to Snedecor & Cochran (1956).

The amount of FSH released after GnRH was calculated by the area under the curve (Thibier, 1976) and referred to as the so-called total response. It is expressed as ng/ml $\times$ 150 min.

Results

FSH responses

The single GnRH challenges were followed by moderate increases in FSH concentrations with maximum values about 2-fold above initial values as illustrated in Text-fig. 1(a) for the challenge at 12 months of age. These initial values were 1.55 ± 0.10 ng/ml and maximum mean value was 2.69 ± 0.12 ng/ml. The high levels were maintained from 15 to 150 min, gradually declining thereafter. As shown in Table 1, the mean total response at 12 months of age was higher than that at 13 months ($P < 0.05$). There was no between-bull effect ($P > 0.05$).

In the second group, the initial mean FSH concentrations were 2.54 ± 0.14 and 2.13 ± 0.09 ng/ml at 12 and 13 months of age respectively. Although there was a trend for a decrease in FSH concentrations (see Text-fig. 1b), the values found in the 5 h after dexamethasone

Text-fig 1. Mean $\pm$ s.e.m. plasma FSH concentrations in bulls after treatment with (a) 0.25 mg GnRH or (b) 20 mg dexamethasone (Dex.) and 0.25 mg GnRH at 12 months of age.

Table 1. Mean ($\pm$ s.e.m.) areas under the FSH curves in response to a single GnRH challenge or a combined dexamethasone-GnRH treatment of bulls according to semen output classification

Age (months)	Semen category	No. of bulls	FSH after GnRH (ng/ml $\times$ 150 min)	No. of bulls	FSH after dexamethasone-GnRH (ng/ml $\times$ 150 min)
12	Good	9	42.73 ± 5.86	12	59.30 ± 6.49
	Medium	9	44.33 ± 3.74	10	51.21 ± 2.87^a
	Poor	7	49.25 ± 3.67	7	80.68 ± 11.48^a
	Mean	25	44.96 ± 2.73^b	29	61.67 ± 4.39^c
13	Good	9	22.16 ± 2.72	12	45.10 ± 3.44
	Medium	9	27.16 ± 3.54	10	47.26 ± 2.44
	Poor	7	32.87 ± 7.26	7	61.88 ± 18.18
	Mean	25	26.72 ± 2.49^b	29	49.89 ± 4.63^c

For values with similar superscripts: $P < 0.05$.

injections and preceding the GnRH challenge were not significantly diminished. Mean FSH levels increased after GnRH injection in a similar manner on both occasions and as described above, i.e. a ~ 2-fold increase in maximum values. The only difference in FSH profile compared to that seen after the single GnRH challenge was that maximal values in dexamethasone-treated bulls were reached 2 h after GnRH injection, later than when GnRH only was given. However, a significant decline began 2.5 h after the GnRH injection. The mean area under the curve (Table 1) was again significantly higher at 12 than at 13 months of age ($P < 0.05$).

The analysis of variance of the total FSH response after the combined dexamethasone-GnRH treatment revealed a significant individual effect and the computed intra-class correlation was 0.45 ($P < 0.05$). The FSH responses to GnRH or dexamethasone-GnRH did not appear to be correlated to the LH or testosterone response in the same samples and reported earlier (Abdel Malak & Thibier, 1982).

Relationship between the total FSH responses and the semen output characteristics

The results of the total FSH responses were significantly different ($P < 0.05$) only after dexamethasone-GnRH injections at 12 months of age when the poor and medium semen-producing groups were compared. This was not found again at the following test, 1 month later. The linear correlations between FSH response to dexamethasone-GnRH and the semen output characteristics were negative and mostly significant ($P < 0.05$) (Table 2). The total sperm abnormalities, the percentages of abnormal heads and decapitated spermatozoa were also significantly correlated to FSH responses (r ranged from 0.39 to 0.69; $P < 0.05$).

Table 2. Coefficients of correlation between the FSH response after dexamethasone-GnRH and sperm output characteristics in 29 Montbéliarde bulls

	12 months of age	13 months of age
Concentration (10^9 spermatozoa/ml)	NS	-0.39
Total number (10^9 spermatozoa)	NS	-0.38
% of motile spermatozoa	-0.39	-0.45
% of total sperm abnormalities	0.53	0.49
% of head abnormalities	0.39	0.55
% of decapitated spermatozoa	0.69	0.43

N.S., $P > 0.05$.

Discussion

The FSH response to a GnRH challenge was more moderate than that of LH in terms of magnitude of release and this is quite consistent with what has been previously reported for normal (Schanbacher & Echternkamp, 1978), cryptorchid (Schanbacher, 1978) or hemicastrated bulls (Barnes *et al.*, 1981). This difference is thought to be essentially related to the distinct qualitative and quantitative feed-back effects of testicular steroids and inhibin (Amann & Schanbacher, 1983). It is unclear why mean total responses after both types of treatments were higher at 12 months than at 13 months of age, although it may relate to continuing maturation of the sperm-producing systems and associated regulatory mechanisms. However, the time sequence of release is similar for both gonadotrophins. Both LH and FSH reached their maximum values later when dexamethasone was injected before GnRH. The LH response was shown to be related to the depressive

effect of dexamethasone on pituitary sensitivity to release this gonadotrophin (Chantaraprateep & Thibier, 1978; Thibier, 1978). To our knowledge, this has not been documented for FSH. Although there is a tendency for FSH concentrations to decline 3–5 h after dexamethasone injection, the lack of significant difference in these mean values precludes any definite conclusion.

However, dexamethasone clearly has an effect on FSH release because there was significant intraclass correlation within individuals (repeatability) after the combined dexamethasone–GnRH treatment which was not observed after a single GnRH challenge. This shows that such a combined treatment is capable of revealing an individual repeatability in both LH and FSH responses whereas GnRH challenge alone could only show a smaller and moderate intraclass correlation coefficient for LH (Abdel Malak & Thibier, 1982) and no correlation for FSH in the data reported here. Although such repeatability could be found, it was not possible to discriminate significantly amongst the 3 categories of bulls using LH data (previous report) or FSH responses (present study). The fact that this was observed after a single GnRH injection is consistent with the above-mentioned lack of FSH repeatability. After the dexamethasone–GnRH combined treatment, a significant difference was found only between medium and poor semen producers and then only at the 12 months challenge. It seems rather illogical that only medium and not good producers are significantly different from the poor group, and as this difference was not found on the second occasion, this may be a random non-specific effect. On both occasions, however, the poor semen-producing group had the highest mean FSH concentrations after the dexamethasone–GnRH challenge. This is in agreement with the findings of the negative relationship between the magnitude of the FSH response and the quality of the spermogram. The above-mentioned observation might therefore be due to use of insufficient numbers of bulls to distinguish between the 3 categories of animals.

The most striking feature of this study, in addition to the significant repeatability of FSH responses, was the negative linear correlation between the release and the quantitative and qualitative characteristics of semen output. This is in good agreement with studies of men which have also revealed negative correlations between total sperm counts and urinary FSH (Christiansen, 1975) or spontaneous plasma profiles of FSH (Hopkinson, Mauss, Schenk, Frite & Hirschmauser, 1977; Courtant & Comhaire, 1978). However, we have demonstrated that even in azoospermic bulls the spontaneous profiles of FSH are not able to reveal any variation or impairment in semen output except in the Klinefelter's syndrome (Abdel Malak & Thibier, 1984; Abdel Malak *et al.*, 1984) and it is only after such a dexamethasone–GnRH combined stimulation of FSH that such a relationship may be seen. Furthermore, the present study shows, for the first time to our knowledge, that qualitative criteria of semen such as motility are negatively correlated while morphological abnormalities are positively correlated to the magnitude of the FSH response. This is also in good agreement with the report of a drastic alteration in the percentage of live spermatozoa or in their motility after passive immunization against FSH in the male monkey (Murty, Sheela, Rani, Moudgal & Prasad, 1979).

In conclusion, we have demonstrated that the magnitude of the pituitary ability to release FSH was an individual characteristic only after the combined dexamethasone–GnRH injections. The magnitude of this response was negatively correlated with the individual semen output characteristics such as total sperm and percentage motility but positively correlated with the incidence of abnormal spermatozoa. However, it is unclear whether this could be used for an early selection of young bulls for their future ability to produce semen of good quality. Nevertheless the pretreatment with dexamethasone appears to offer a significant improvement over the simple GnRH challenge in studying FSH capacity and semen production characteristics.

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