Onset of puberty 
in Maremmana heifers

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ABSTRACT

The Maremmana breed is characterised by rusticity, resistance to harsh environments and good growth ability and it could be considered as one of the most suited breeds for extensive rearing in Italy. Moreover, Maremmana presents high fertility and good fostering ability but, on the other hand, the age at first calving appears quite high. This could probably be due to reduced feed availability to which animals are frequently subjected. The aim of this paper was to determine the age at puberty in Maremmana heifers, the repeatability of the reproductive cycles along the seasons and to verify the possibility to anticipate the age at first service. Haematic levels of progesterone and 17-β-estradiol were determined weekly in 6 heifers raised in pens and fed with 0.70 Milk FU/kg DM and 121 g of gross protein/kg d.m, starting from 9 up to 25 months of age. Metabolic profile was determined monthly to highlight possible metabolic unbalances that could affect reproductive activity.

Plasma was submitted to RIA analysis in order to measure steroidal hormones. Puberty was considered reached when two subsequent measures of progesterone resulted higher than 1 ng/ml of plasma. This happened in 5 out of 6 heifers at an average age of 17 months. The fluctuation of the hormonal levels throughout the year seems to exclude the seasonality of the oestrous cycle. The results showed that the level of the most important metabolites is within the normal range and it does not seem to interfere with the choice to anticipate the first service by one year. Possible periods of anoestrus noticed in some herds could be linked to the reduced availability of pastures in a few months of the year.

Key words: Maremmana, Puberty, Metabolic profile, Hormones.
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La riproduzione può essere dovuta al tardivo raggiungimento di un adeguato sviluppo corporeo a causa delle spesso critiche disponibilità alimentari cui le bovine sono sottoposte. Il sistema di allevamento tradizionale è basato infatti sul pascolo, solo eccezionalmente integrato con fieni di produzione locale. La Maremmana è caratterizzata anche da un’accentuata concentrazione dei parti nei mesi primaverili e questo, unitamente alla ridotta attività gonadotropica nei mesi invernali precedentemente riscontrata, ha indotto ad ipotizzare una sorta di stagionalità riproduttiva, con anestro autunno-invernale, peraltro insolita nella specie bovina.

Scopo del presente lavoro è stato determinare il momento della pubertà, valutare il verificarsi ed il ripetersi dei cicli riproduttivi nel corso dell’anno e verificare la possibilità di anticipare di un anno l’inizio della carriera riproduttiva di bovine Maremmane. Su 6 bovine allevate in condizioni ambientali non critiche, stabulate ed alimentate con razioni contenenti 0,70 UFL/kg SS e 121 g di proteina grezza/kg SS, sono stati determinati settimanalmente, da 9 a 25 mesi di età, i livelli ematici di progesterone e 17-β-estrodiolo e mensilmente quelli dei principali metaboliti. Per il dosaggio degli ormoni steroidei il plasma è stato sottoposto ad analisi RIA. Per la definizione del profilo ematico, dopo misurazione dell’ematocrito con microcapillari eparinizzati posti in centrifuga a 3600gpm per 5 minuti, sono stati determinati, tramite analisi colorimetrica con spettrofotometro e reagenti Sclavo-Diagnostics-Int., sul plasma: glucosio, proteina totale, albumina, colesterolo totale e cloro; sul siero: calcio, fosforo inorganico e magnesio. La pubertà, considerata raggiunta quando il livello di progesterone si è mantenuto nel plasma, in due previe consecutivi, superiore ad 1 ng/ml, si è manifestata, su 5 bovine, ad un’età media di 17 mesi. Il diagramma delle oscillazioni dei tassi ormonali nel corso dell’anno sembra escludere la stagionalità dei cicli estrali. I valori stimati dei metaboliti più importanti riscontrati a fine prova, quando le bovine avevano circa 2 anni di età ed un peso superiore ai 400 kg, sono in linea con i valori normali tabulati per bovine adulte (Ematocrito: 45%; Glucosio: 4,052 mmol/l; Proteina totale: 80,612 g/l; Calcio: 1,973 mmol/l; Fosforo: 1,724 mmol/l) e non sembrano tali da interferire negativamente con la scelta di anticipare l’immissione alla monta di un anno. Eventuali periodi di anestro riscontrabili nelle mandrie sono da mettere in relazione alla scarsa offerta dei pascoli in alcuni mesi dell’anno che non riesce a sopperire ai fabbisogni nutritivi delle mandrie.

Parole chiave: Maremmana, Pubertà, Profilo metabolico, Ormoni.

Introduction

The quality of Maremmana beef cattle meat is well known (Giorgetti et al., 1996; Bozzi et al., 1998; Sargentini et al., 1999), but little information is available about the reproductive behaviour of this breed. It is characterised by an extremely low percentage of stillborn calves, good maternal aptitude, fairly good milk production and reduced frequency of difficult parities. But on the other hand it presents an average age at first calving of 3 years and 9 months (J annella et al., 1977), only slightly anticipated in recent years (Ronchi and Nardone, 1988). Some herds present fertility indices lower than expected. Finally the Maremmana breed is characterised by strong seasonality of parities, gathered during the spring period. This behaviour is undoubtedly favoured by the breeding technique but it could also be an index of oestrus seasonality, though not so common in cattle. During the winter period Maremmana calves and heifers seem to show an almost null level of estrogens and a low level of progesterone (Santarelli, 1988): this behaviour leads to hypothesize a strongly reduced gonadotropic activity, with missing heats and autumn-winter anoestrus. Some authors (Lucifero, 1978; Lucifero et al., 1981; Giorgetti et al., 1983, 1985, 1986) attribute the reason for these problems to the oscillating and often critical
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Feed availability to which animals assigned to reproduction are submitted. In particular, low levels of energy and unbalanced Ca/P ratio with a strongly marked phosphorus deficiency during some years and seasons were considered responsible for these problems. The study of Metabolic Profile (M.P.) was originally used for dairy cattle as an investigation tool to prevent the onset of some “production diseases” but it could also be useful in beef cattle in pointing out the presence of sub clinical alterations linked to the animals’ nutrition that could negatively affect the reproductive function (Lucifero et al., 1978; Bittante et al., 1980). In fact, the unbalance between feed intake and losses linked to maintenance, production and reproduction produces changes in haematic concentration of some parameters. Thus, the observation of these changes could help to point out deficiency status or metabolic unbalances before they appear in the form of illness in the animal or reduced reproductive efficiency. The significant presence of estrogens and progesterone in the blood is instead an index of the onset of the reproductive period (puberty) and the changes in their ratio characterises the follicular and the progestinic phases of the oestrus cycle.

The aim of the present study was to verify the onset of puberty and the winter seasonality in Maremmana heifers.

Material and methods

Six Maremmana heifers, born during the spring period, at an average age of 9 months (281 ± 17d) and at an average live weight of 200 kg (196.8 ± 21.6 kg) were raised in pens, where they remained to 2 years of age (750 ± 17 d). Two years could be considered an optimal age to start the reproductive activity of the heifers. The animals reared on free range were fed with a diet suited for growing females so that the average daily gain could be around 0.600 kg. Such a diet with 0.70 Milk F.U./kg d.m. and 121 g of crude protein/kg d.m., was composed of 65-70% of alfalfa and mixed hays, 35-30% of concentrates (80-84% maize and barley) and integrated with mineral elements (licking stone). Heifers were weighed weekly and submitted to a withdrawal of blood to determine the levels of progesterone and 17-beta-estradiol. Blood samples were collected from the jugular vein with a 10ml heparinised Vacutainer tube. Plasma was recovered with centrifugation at 3500 rpm and then frozen to -20 °C. Samples were submitted to radio immuno assay technology (RIA) at the laboratories of Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d’Aosta (Torino).

The analyses were implemented with a commercial RIA kit based on competition between standard and radio labelled analytes for a fixed and limited number of anticorpal sites.

The 17-beta-estradiol was determined with a double anticorpal method. The radioactivity of the sediment was measured by a Cobra 5003 Packard gamma counter. The signal was automatically interpolated on a standard curve with concentration between 100 and 1000 pg/ml directly obtaining the hormone concentration. Progesterone dosage was obtained using a coated-tube with a direct count of the bound radioactivity. Concentrations of progesterone (ng/ml) were directly recovered interpolating the signal on a standard curve scaled from 0.1 to 60 ng/ml. Both analytical methods are officially recognized and usually employed to determine such hormones in bovine blood. Puberty was considered achieved when the progesterone level was up to 1ng/ml of plasma in two consecutive withdrawals (Bergfeld et al., 1995).

Concurrent with the aforementioned withdrawal, heifers were subjected monthly to a withdrawal of blood to determine the metabolic profile. Blood samples were collected from
the jugular vein with a 10ml EDTA coated Vacutainer tube. Plasma and serum were recovered with centrifugation at 3500 rpm and then frozen to -20 °C. Samples were submitted to colorimetric analysis. The following analyses were performed: glucose (GLU) with oxidase/peroxidase colorimetric determination; Total protein (TP) with biuret-tartrate colorimetric determination; Albumin (ALB) with BCG colorimetric determination; Total Cholesterol (TCho) with enzymatic colorimetric determination; Calcium (Ca) with o-Cresolphthalein colorimetric determination; Inorganic phosphorus (IP) with phosphomolibdate colorimetric determination; Magnesium (Mg) with xylidine-Blue colorimetric determination; Chloride (Cl) with Mercury Thiocyanate colorimetric determination. Globulin were calculated as (total protein - albumin). Ca, IP and Mg were determined on serum whereas GLU, TP, ALB, TCho and Cl were determined on plasma. PCV value was obtained using capillary tubes after centrifugation at 3500 rpm for 5'.

Data were submitted to statistical analysis using the REG procedure of SAS (1993) up to the higher level of significance. Least square means were derived from regression equations. The number of the reproductive cycles occurring along the trial was calculated and their length was submitted to ANOVA following GLM procedure of SAS (1993) in order to determine the cyclicity and the regularity of oestrous. Finally, correlations among weight, age and haematic parameters were calculated using the CORR procedure of SAS (1993).

**Results and discussion**

Five out of 6 heifers reached puberty at around 510 days of age (17 months) and an estimated live weight of 320.4 kg (Table 2), quite far from the value of 12-13 months reported for dairy cattle (Fajersson et al., 1991) and also far from the 13 months found by Bergfeld et al. (1995) and from the 12-13 months found by Patterson et al. (1992) both working on beef cattle.

Lower ages of puberty have been reported for zebu x dairy cattle, zebu x beef cattle and East African zebu (Galina and Arthur, 1989). Higher values have been reported for Sahiwal females (Thair et al., 1983), Ongole zebu (Rao and Rao, 1981), Brahman (Plasse et al., 1968) and Criollo cattle (Linares et al.,

| Table 1. Mean value of live weight and age and correlation coefficients between live weight, age and blood parameters. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | Age | PCV | GLU | TP | Alb | GL | Ca | IP | Mg | Cl | Tcho |
| ±SD | d | % | mmol/l | g/l | g/l | g/l | mmol/l | mmol/l | mmol/l | mmol/l | mmol/l |
| Weight | 332.6 | ± 88.3 | 0.962 | 0.163 | 0.035 | 0.435 | 0.478 | 0.310 | -0.563 | 0.181 | 0.140 | -0.153 | 0.673 |
| Age | 503.7 | ± 34.6 | 0.172 | 0.020 | 0.434 | 0.441 | 0.323 | -0.587 | 0.182 | 0.153 | -0.133 | 0.622 |

** Probability value, P<.01.

PCV = Packed Cell Volume; GLU = Glucose; TP = Total Protein; Alb = Albumin; GL = Globulin; Ca = Calcium; P = Inorganic Phosphorus; Mg = Magnesium; Cl = Chloride; Tcho = Total cholesterol.
Similar ages at puberty were reported in Holstein cross, Jersey x Sahiwal (Thair et al., 1983) and Brahman x Shorthorn (Plasse et al., 1968). The reference to the Taurine breeds raised in tropical or sub-tropical environments or even to the Zebuine breeds (Bos indicus) is justified by the fact that reproductive parameters and herd breeding policies of rustic and not well-known breeds are more similar to the former than to those known for specialised breeds.

Figures 1 and 2 report the average level of the steroidal hormones (progesterone and 17-β-estradiol) for the heifers that showed oestrus activities (puberal heifers) and the level for the heifer did not show the oestrus (pre-puberal heifer). Progesterone haematic level of the puberal heifers shows the typical oscillating trend (Figure 1). Considering the number of days that pass from two consecutive progesterone peaks, starting from puberty, it is possible to estimate the length of the cycle and its cyclicity. The trial includes on average 9 cycles linked to the oestrus appearance. Cycles presented an average length of 20.2 days and a regular repetition of them could be hypothesised. Also for the 17-beta-estradiol the puberal heifers show the classic pattern of the steroidal hormones with fluctuating trends (Figure 2). Observing the level of hormones for the puberal heifers (Figures 1 and 2), it could be noted that the oestrus are compatible with the normal reproductive activity of the herds and the heifers could be mated starting from 24 months of age moving up the reproductive career by almost one year. Also the growth performances seem to confirm this possibility. Figure 3 reports the curve describing the regression of live weight on age. The growth of live weight in relation to age follows a cubic trend showing a self-accelerated growth up to the age between 390 and 450 days when the animals weighed on average 282 kg. The following phase makes it possible to reach an estimated weight of over 400 kg at 24 months of age. Thus if we consider that the literature reports a live weight around 600-650 kg for the adult Maremmana females, it seems the goal has been reached having animals that weighed almost 70% of the adult live weight at 24 months of age, a value that is fully

### Table 2. Significant regressions between the haematic parameters and age (x).

| Parameter            | Equation                                   | Sign. | RSD    | R²    |
|----------------------|--------------------------------------------|-------|--------|-------|
| Live weight          | $Y=106.780-0.185x+0.002x^2-0.0000016x^3$   | **    | 23.720 | 0.930 |
| PCV                  | $Y=204.633-0.9368x+0.00162x^2-0.00000085x^3$ | **    | 5.919  | 0.485 |
| Glucose              | $Y=0.985+0.0147x-0.0000145x^2$             | **    | 0.919  | 0.065 |
| Total protein        | $Y=91.124-0.137x+0.00017x^2$               | **    | 8.966  | 0.247 |
| Globulin             | $Y=66.823-0.135x+0.000157x^2$              | **    | 8.124  | 0.189 |
| Albumin              | $Y=84.348-0.401x+0.000850x^2-0.00000056x^3$ | **    | 0.316  | 0.293 |
| Total cholesterol    | $Y=8.450-0.047x+0.000106x^2-0.00000007x^3$ | **    | 0.451  | 0.429 |
| Chloride             | $Y=276.339-1.375x+0.0031x^2-0.000022x^3$   | **    | 14.753 | 0.129 |
| Calcium              | $Y=-0.37+0.01062x-0.00001023x^2$           | **    | 0.199  | 0.436 |
| Inorganic phosphate  | $Y=3.668-0.0027x$                          | **    | 0.570  | 0.338 |
| Ca/P                 | $Y=-0.897+0.00737x-0.00000553x^2$          | **    | 0.300  | 0.402 |

** P<.01.
Figure 1. Average level of haematic progesterone for puberal heifers (solid line) and pre-puberal heifer (dotted line) from 280 to 750 days of age.

Figure 2. Average level of haematic 17-β-estradiol for puberal heifers (solid line) and pre-puberal heifer (dotted line) from 280 to 750 days of age.
compatible with the start of the reproductive career (Acciaioli et al., 1988). Average daily gain calculated as first derivative of the growth curve achieves the value of 0.648 kg similar to what is suggested for cows (INRA, 1988), and in agreement with the formulated diet (see material and methods).

Table 1 reports the correlation coefficients among weight, age and haematic parameters. Weight and age are, as expected, highly correlated (0.962). Both the parameters are positively correlated with total protein, albumin, globulin, cholesterol. High correlations but with a negative sign were found among weight-age and calcium. A higher level of calcium during the first phases of life could easily be explained considering the higher requirements of it for skeletal growth.

Table 2 reports the equation of haematric parameters according to age evolution. All the investigated variables showed significant trends but the determination coefficient is never higher than 50% thus demonstrating a partial relationship between the metabolites and somatic growth. PCV is described by a third order curve with higher values at the start of the trial (53.06%), lower at 450 days (33.7%) and then higher again (45%). High values (over 45%) have been found by Lucifero et al. (1980) in female cattle during the winter period. Glucose presents a parabolic trend showing a growing phase up to 510 days of age when it reached the value of 4.711 mmol/l and then a decreasing trend up to the end of the trial with a final value of 4.052 mmol/l, similar to that found by Giorgetti et al. (1983) in Maremmana cows. It is noteworthy to point out that, as previously observed (Giorgetti et al., 1986), the Maremmana breed presents PCV and glycaemia values higher than other breeds, probably because of neuroendocrinial reasons linked to blood withdrawal stress that is greater in animals characterised by extreme rusticity. Often these two values grow following stress conditions. Total protein presents a quadratic trend and the values obtained could be related to the low
level of globulins during the first period of life (Lupi et al., 1994). Total protein values obtained at the end of the trial (80.612 g/l) were similar to those found in adult females by Giorgetti et al. (1983). While globulins showed a trend similar to total protein with the lowest value at 420 days of age (37.82 g/l), albumins seems to follow a cubic trend, decreasing up to 360 days of age (least square mean: 24.02 g/l), and then growing up to 630 days (29.06 g/l) with a final decreasing period until the end of the trial. Calcium showed a quadratic trend (Figure 4) with ascending period up to 510-540 days of age (2.38 mmol/l). Values found in the present trial result slightly lower than those related by Giorgetti et al. (1986); this could be due to the age of the animals and to the strong mobilisation of this mineral element used in bony tissue formation which is completed within the early years of life. Phosphorus shows a linear trend and decreases when age increases (Figure 4). Estimated values of this element varied from 2.93 mmol/l at 270 days of age (9 months) to 1.72 mmol/l at the end of the trial. These values could be related to the metabolic differences along the various phases of body growth as already stated by Calamari et al., (1982). Calcium/phosphorus ratio (Figure 4) presents quadratic trend with a peak of 1.58 at 660 days of age and then a decreasing phase. On the importance of this ratio it is important to bear in mind that for the first element feed deficiencies or excesses do not lead to substantial haematic variations due to the great body reserves and to the hormonal control, whereas the haematic level of inorganic phosphorus is strictly linked to the feed availability of such element (Forar et al., 1982); thus wide variations of this ratio in the feed could be easily counterbalanced by the animals (Guéguen, 1978).

Figure 4. Evolution of haematic level of calcium, phosphorus and Ca/P ratio according to age.
Conclusions

The regular gonadotropic activity during the months following puberty makes it possible to discard the hypothesis of winter anoestrus. The performances obtained at 2 years of age are strictly connected to feeding regime: since the reproductive potential even during the winter has been verified and discarding the hypothesis of an anoestrus due to temperature and/or photoperiod, the reduced gonadotropic activity has to be related solely to the low availability of food and, above all, to the low quality of it immediately after the mating period. Particular attention should be given to feed integration during the winter period. Finally, the breeder has to assure the fulfilment of the nutritive requirements even during the periods of low pasture availability in order to be able to put the heifers for mating at 24 months of age.

Metabolic profile evidenced a haematic outline that, with few deficiencies for some parameters, could be interpreted as a classical pattern of the animals’ physiological evolution related to age. The weight achieved at the end of the trial of around 400 kg, equal to 70% of the adult weight, and the level of the main blood metabolites seems to suggest the possibility to anticipate the age at first service by one year.

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