CARYOLOGIA Vol. 56, no. 2: 205-211, 2003

An improved characterization of horse (*Equus caballus*, 2*n*=64) chromosomes by using replicating G and R banding patterns

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Abstract - Peripheral blood lymphocytes were cultured and treated for early- and late-BrdU incorporation to perform replicating G- and R-banding patterns, respectively. Slides were treated for GBG-, RBA- and RBG-banding techniques. Improved banded karyotypes at early- (350 bands) and pro-metaphase (500 bands) stage were performed and GBG- and RBA-banded prometaphase karyotypes were presented for the first time on this species. All chromosomes, including the small acrocentrics, show clear and distinguishable G- and R-banding patterns. Chromosome identification followed the latest chromosome standard nomenclature (ISCNH 1997). This study is also our contribution to further standard karyotype attempts at the prometaphase stage.

Key words: horse, cytogenetics, G-bands, R-bands.

INTRODUCTION

Horse chromosomes (ECA) have previously been studied by using both G- and R-banding techniques (MACHIULIS *et al.* 1984; RICHER and ROMAGNANO 1985; ROMAGNANO *et al.* 1987; POWER 1990; RONNE *et al.* 1993) and standard karyotypes have been proposed at different degrees of chromosome contraction (READING CONFERENCE 1980; RICHER *et al.* 1990; ISCNH 1997). In particular, GTG and RBG-banded karyotypes and relative ideograms at the 436 bands were proposed in the latest standard karyotype (ISCNH 1997). However, several acrocentric chromosomes (22, 26, 27, 28 and 29) show similar banding patterns, especially when GTG-banding was applied (ISCNH 1997). Some biarmed chromosomes (ECA8p and ECA10p) also show similar GTG-banding patterns (ISCNH 1997).

Early and late incorporation of BrdU allow replicating G and R banding patterns, respectively, to be obtained which are exactly complementary. At the same time, replicating G-bands are very similar to the GTG-banding, allowing the establishment of corresponding GTG-banding to each R-banded chromosome pattern. This approach has successfully been applied in river buffalo (IANNUZZI *et al.* 1990), sheep (IANNUZZI et *al.* 1995), goat (IANNUZZI *et al.* 1996) and cattle (IANNUZZI 1996) chromosomes, allowing a detailed description of single chromosome and ideograms. These studies were also useful when G and R-banded standard karyotypes for river buffalo (CSKBB 1994), cattle, sheep and goat (ISCNDB2000 2001) were performed.

In the present study, improved characterization of horse chromosomes was achieved by using early and late incorporation of BrdU. Further-

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more, this is the first report where GBG- and RBA-banded karyotypes of horse at the 500-band stage were constructed according to the ISCNH (1997). This study is also our contribution to further standard karyotype processes.

MATERIALS AND METHODS

Material

Two sets of cell cultures from peripheral blood samples of 12 horses (8 males and four females) were set up. One ml of whole blood sample was cultured for about 72 h in 8 ml of RPMI 1640 medium enriched with inactivated foetal bovine serum (1 ml), Pokeweed mitogen (7 µg/ml, Sigma), Penicellin-streptomicin (0.1 ml) and 5 µl of sodium heparin.

Cell cultures were treated for early- (G-bands) and late- (R-bands) incorporation according to the two following protocols (and cultures).

Early BrdU incorporation

Simultaneous adding of BrdU (20 µg/ml) and MTX (0.5 µg/ml) was performed 20-22 h before harvest in order to obtain partial cell cycle block in S-phase and early BrdU incorporation, as reported previously in other species (IANNUZZI *et al.* 1989). Cells were washed once 15-17 h later with Puck's saline solution to remove BrdU and MTX and recovered for 5.5 h in fresh RPMI medium containing thymidine (10 µg/ml).

Late BrdU incorporation

After about 48 h of cell grow, thymidine block (300 µg/ml) was performed for 15-17 h. Then cells were washed once with Puck's saline solution and recovered for 6.5 h in RPMI medium containing BrdU (20 µg/ml) and Hoechst 33258 (40 µg/ml) and 5µl of sodium heparin.

Colcemid treatment (20 µl for 30 min and 70 µl for 1,5 h when early and late BrdU-incorporation was achieved, respectively), hypotonic treatment (0.5% KCl for 20 min at 37° C) and four fixations (the third overnight) in acetic/methanol 1:3 followed.

GBG- and RBG-banding

Slides treated for early (G-bands) and late (R-bands) BrdU incorporation and one week old (or more) were stained with Hoechst 33258 (25 µg/ml in distilled water) for 10 min, then washed with distilled water and mounted with 0.8 ml of 2xSSC (pH=7.0) by using a glass coverslip (without pressure). Slides were then exposed to UV light for 30-60 min at the distance of 4 cm from the lamp, washed with distilled water, treated in 2xSSC at 60° C for 30 min, rinsed in 2xSSC at room temperature, washed with distilled water, air dried and finally stained with Giemsa (8% in phosphate buffer pH=7.0) for 30 min. Slides were observed without coverslips. This procedure is very useful when slides need to be sequentially treated for other banding techniques (C-banding, Ag-NOR staining).

RBA-banding

Slides were stained with Hoechst 33258 and exposed to UV light as reported for RBG-banding. After washing with distilled water, slides were stained with acridine orange (0.01 % in phosphate buffer pH=7.0) for 10 min. Slides were then washed with tap and distilled water, air dried and mounted after one hour in phosphate buffer and a day later (or more) observed at the fluorescent microscope.

All karyotypes were constructed by using single cells and following the latest international chromosome nomenclature of the horse (ISCNH 1997).

RESULTS AND DISCUSSION

Figs. 1 and 2 show GBG-banded horse karyotypes at the early-metaphase (350 bands) and pro-metaphase (500 bands) stage. The banding achieved is very clear and all chromosomes are easily identifiable. A GBG-banded karyotype has previously been reported by RICHER and ROMAGNANO (1985). However, the resolution was much lower compared to that we performed in the present study (both at early- and pro-metaphase stage). Furthermore, the G-banded karyotype reported by the above authors was not in agreement with the standard karyotype published later (ISCNH 1997).

Fig. 3 shows the RBA-banded horse karyotype at the 500-band stage from a male horse pro-metaphase cell. The Y chromosome is almost all R-band positive but the intensity of fluorescence is lower than that of the autosomal R-band positive regions. The same behaviour was observed when using GBG-banding, although the intensity of staining is more evident at the telomeric regions, as occurs when GTG-banding is applied (ISCNDH 1997). This different behaviour between structural G-banding (GTG technique) and replicating banding (both GBG and RBA/RBG banding) on the Y chromosome may arise from the incorporation of BrdU. Indeed, clear R-bands negative (and G-band positive) can be seen along the Y chromosome, in addition to that located at the telomere (R-band negative /G-band positive), when using RBA- or GBG-banding (Figs. 2 and 3).

RBG-banding patterns (not shown in the present study) agree with the RBA-banding ones and are essentially in agreement with those reported

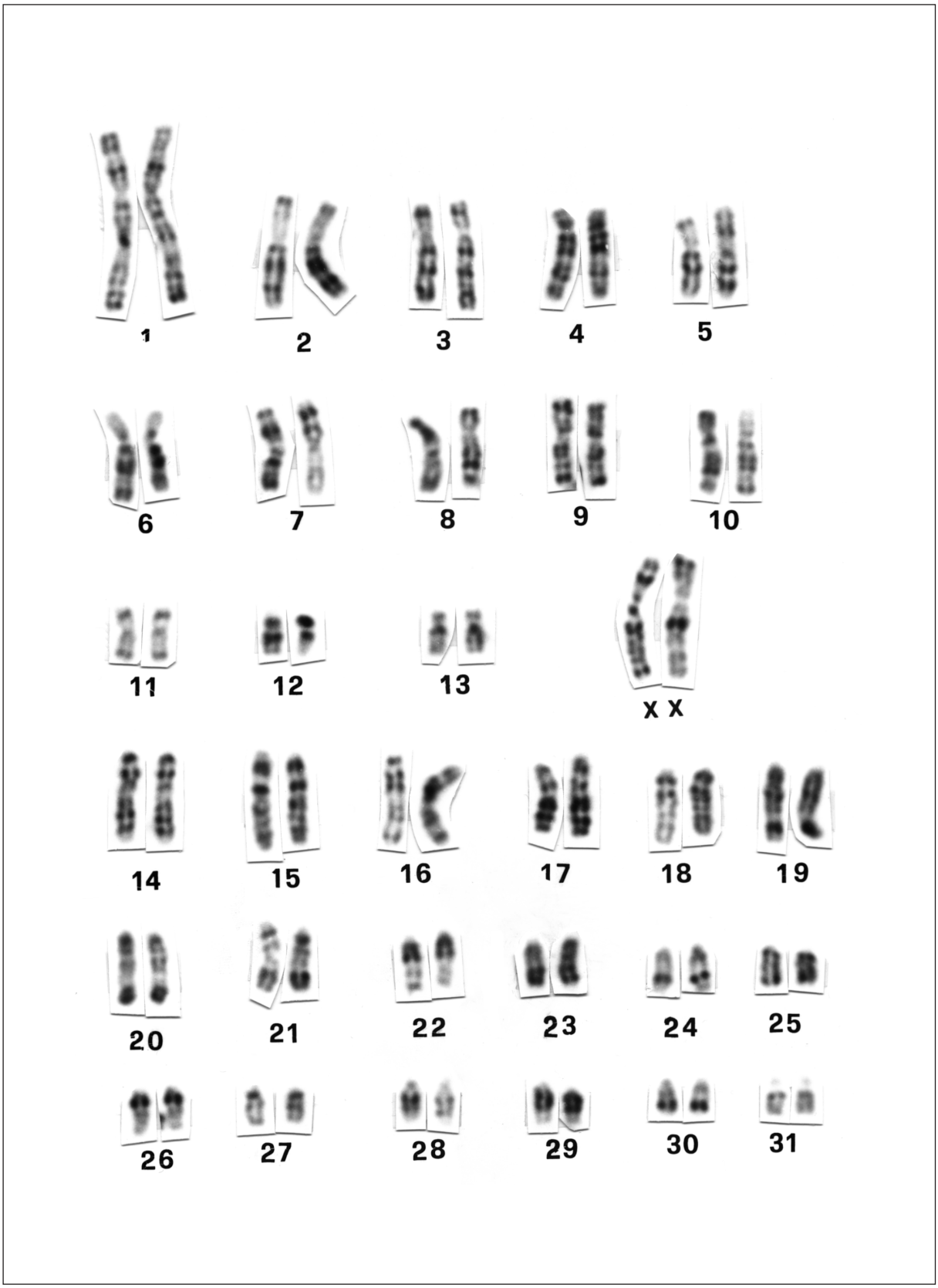

Fig. 1 – GBG-banded female horse karyotype drawn from a single early-metaphase cell (350 bands). Note the clear banding patterns in all chromosomes, including the small acrocentrics.

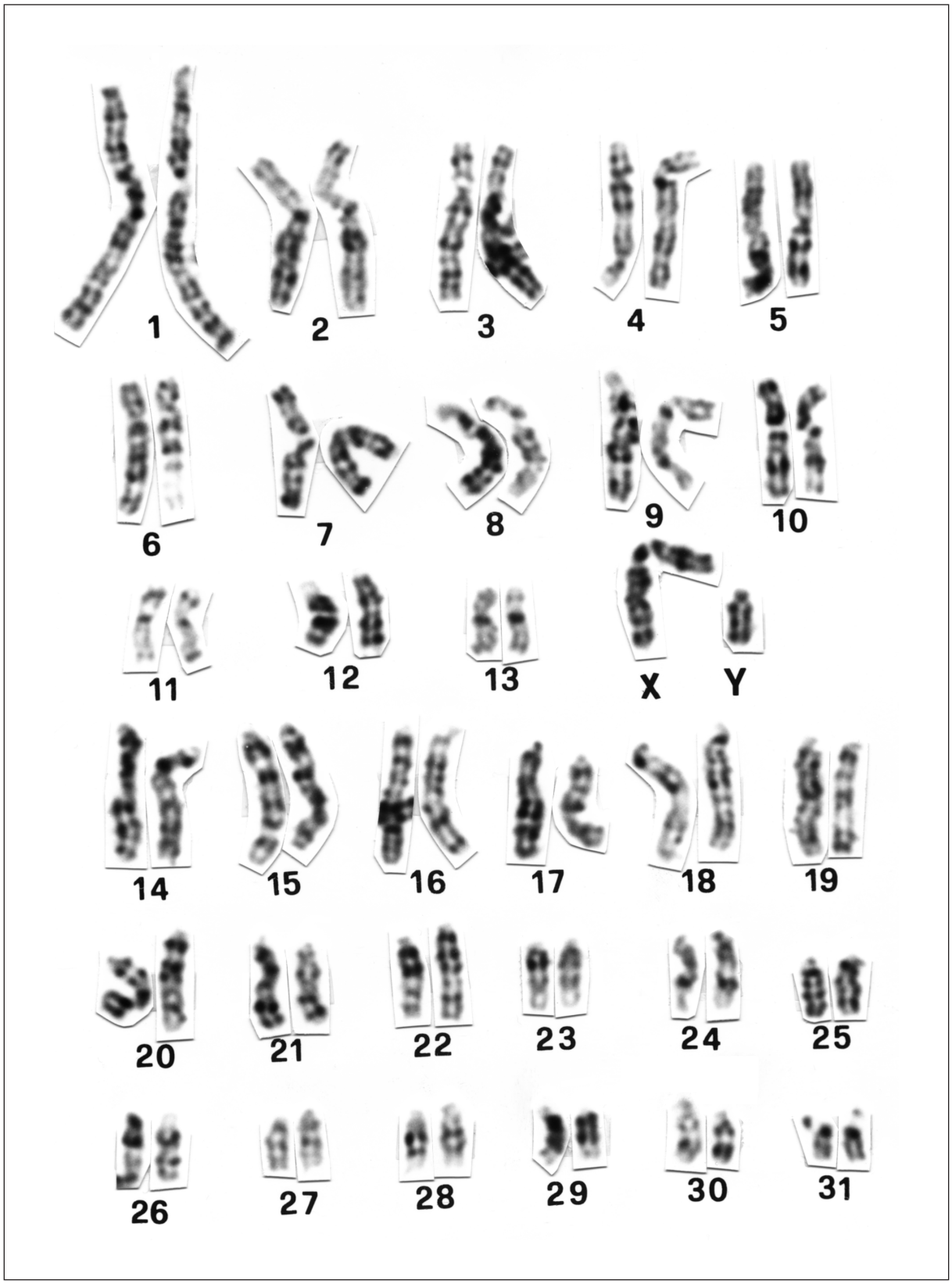

Fig. 2 – GBG-banded male horse karyotype drawn from a single pro-metaphase cell (500 bands). Note the detailed G-banding patterns in all chromosomes. The Y chromosome appears entirely stained with three evident G-bands positive, proximally, centrally and telomerically located.

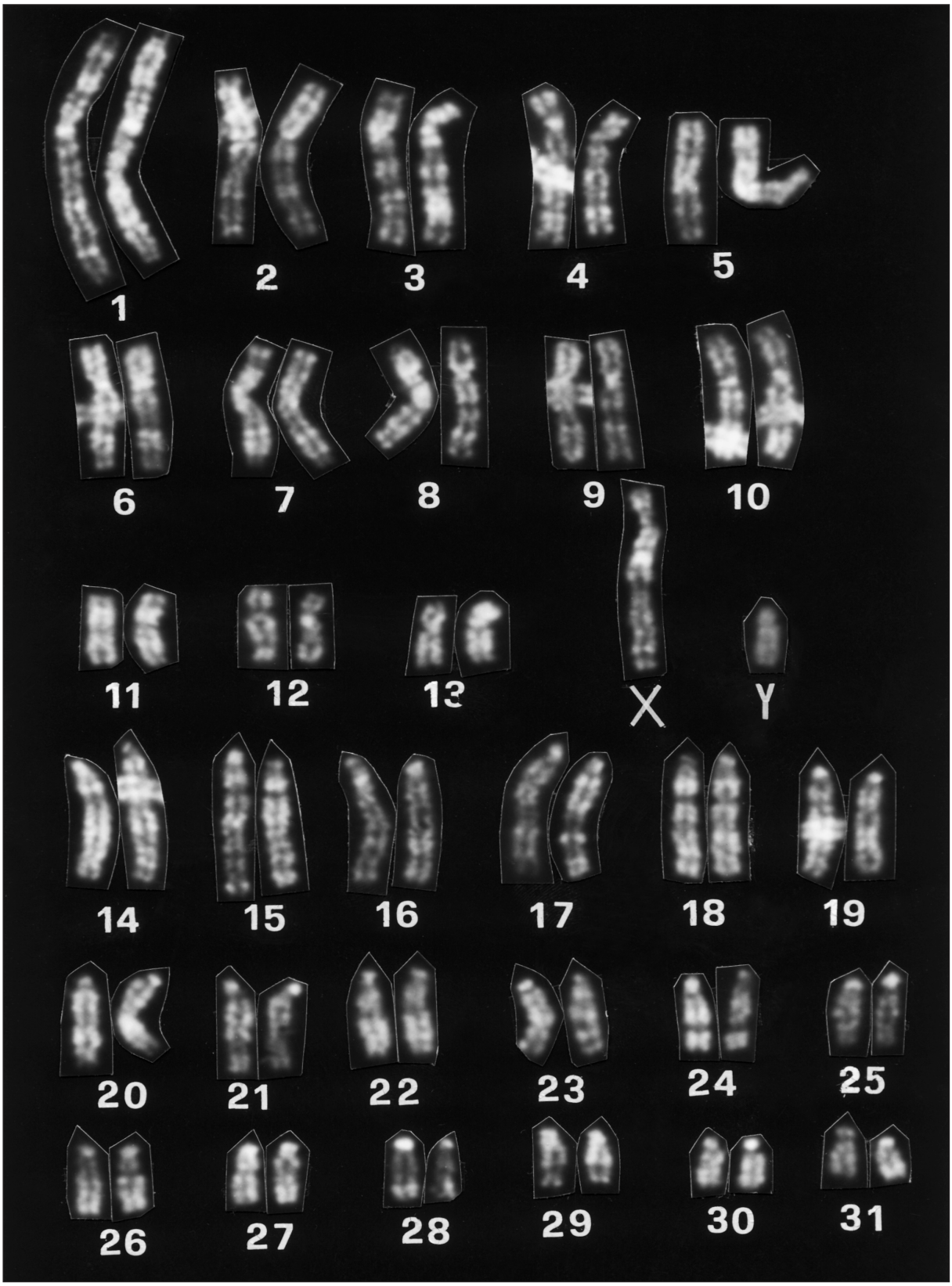

Fig. 3 – RBA-banded male horse karyotype drawn from a single prometaphase cell (500 bands). Note the clear and detailed R-banding patterns in all chromosomes. The Y chromosome appears dull florescence with two more evident bands, proximally and distally located.

by RONNE *et al.* (1993). Only ECA28 shows some apparent difference on the proximal regions. Indeed, RBA-bands 12 and 14 (see on the ISCNH 1997 standard karyotype) appear smaller compared to those achieved when using RBG-banding (RONNE *et al.* 1993; ISCNH 1997). This facilitates the distinction between ECA28 and ECA27 which show very similar RBG-banding (RONNE *et al.* 1993; ISCNH 1997).

G- and R-banding patters achieved in the present study were exactly complementary, allowing easy identification of each chromosome pair and detailed visualization of both G and R-banding in all chromosome regions. Several chromosomes, reported G-band negative at the telomeres with GTG-banding (ISCNH 1997), are clearly GBG-band positive (and R-band negative): 1p, 1q, 2p, 3p, 6q, 11q, 12q, 22. These aspects are important when standard ideograms are performed. Furthermore, the use of GBG-banding, in combination with both replicating R-banding (RBA- and RBG-banding) and GTG-banding allows more precise chromosome ideograms to be constructed. The same approach was employed in cattle, river buffalo, sheep and goat (IANNUZZI 1996; IANNUZZI *et al.* 1990, 1995, 1996, respectively) and when river buffalo (CSKBB 1994), cattle, sheep and goat (ISCNDB2000 2001) standard ideograms were arranged.

In conclusion, simple and repetitive replicating G- and R-banding were achieved in horse chromosomes, allowing further advances in our knowledge of the chromosomes of this important species. Furthermore, GBG and RBA-banded pro-metaphase karyotypes at the 500 band stage were presented for the first time by adding more data on horse karyotypes among those available by using other banding techniques at different degrees of chromosome contraction (MACHIULIS *et al.* 1984; ROMAGNANO and RICHER 1985; RICHER and ROMAGNANO 1985; ROMAGNANO *et al.* 1987; POWER 1990; RONNE *et al.* 1993; ISCNH 1997).

The karyotypes presented here could be particularly useful in both clinical and molecular cytogenetics of the horse since many labs use replicating banding (in particular R-banding), especially when using the FISH-technique (fluorescence R-banding).

This study is also our contribution to further standard karyotype attempts, especially to obtain higher banding pattern resolution, more precise G- and R-banded ideograms, and GBG- and RBA-banded standard karyotypes.

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Received December 10, 2002; accepted February 2, 2003