High throughput RNA isolation from bovine endometrial epithelial cells using the InviMag® Universal RNA Mini Kit on the KingFisher® Flex

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ABSTRACT

Optimal reproductive performance is essential for the survival of each species and the economic basis for dairy enterprises. Implantation and maintenance of pregnancy are critical phases after fertilization and can be affected by several factors including the expression of cytokines, hormones, interleukins and prostaglandins. In order to investigate the mRNA expression pattern of fertilization relevant factors under multiple experimental conditions, it is necessary to adopt an efficient and reproducible system for high throughput RNA isolation. mRNA quantification via real-time RT-PCR includes potential sources of error, which can lead to incorrect results or to variation in the expression level. A detection of the RNA integrity (RIN) helps to standardize the RNA quantification and reflect the RNA quality. RNA purification from hundreds of samples often represents a bottleneck in sample analysis. The use of an automated, easy-to-handle method is therefore highly desirable to increase productivity and reproducibility. In this study we report the successful application of the InviMag® Universal RNA Mini Kit on the KingFisher Flex 96 from Thermo Scientific in order to investigate the expression of different factors playing a key role for fertilization.

METHODS

Oviducts were collected from the slaughterhouse as previously described (1). Due to the fact that experiments with animals should be avoided or replaced by *in vitro* approaches, establishment of cell cultures models were introduced. For the RNA isolation cells after first passage were cultured to confluence in 6 well plates and exposed to media with different nutrient compositions for 12 h, 24 h or 48 h, respectively.

Highly pure total RNA was isolated using the InviMag® Universal RNA Mini Kit on the KingFisher Flex 96. The KingFisher technology is based on magnetic rods transferring particles through the various purification phases. The lysis step is done outside the workstation and the carrier bound DNA is removed by centrifugation. The RNA containing supernatant is transferred to the KingFisher deep well plate and ethanol as well as magnetic particles are added followed by automated RNA purification on the KingFisher platform. The preparation time for 96 samples in parallel takes approx. 1 h – 1 h 30 min.

RESULTS

These kit performances allow the isolation of highly pure RNA without any DNase digestion step.

1) The high purity of the isolated RNA is shown in a RIN Factor of about 10.0.

2) The yield of total RNA was between 10 - 20 µg per well depending on the animal and growing rate.

3) The ratio A_{260}: A_{280} is in average 2.05 (standard deviation +/-0.2%) measured by a NanoDrop photometer**.**

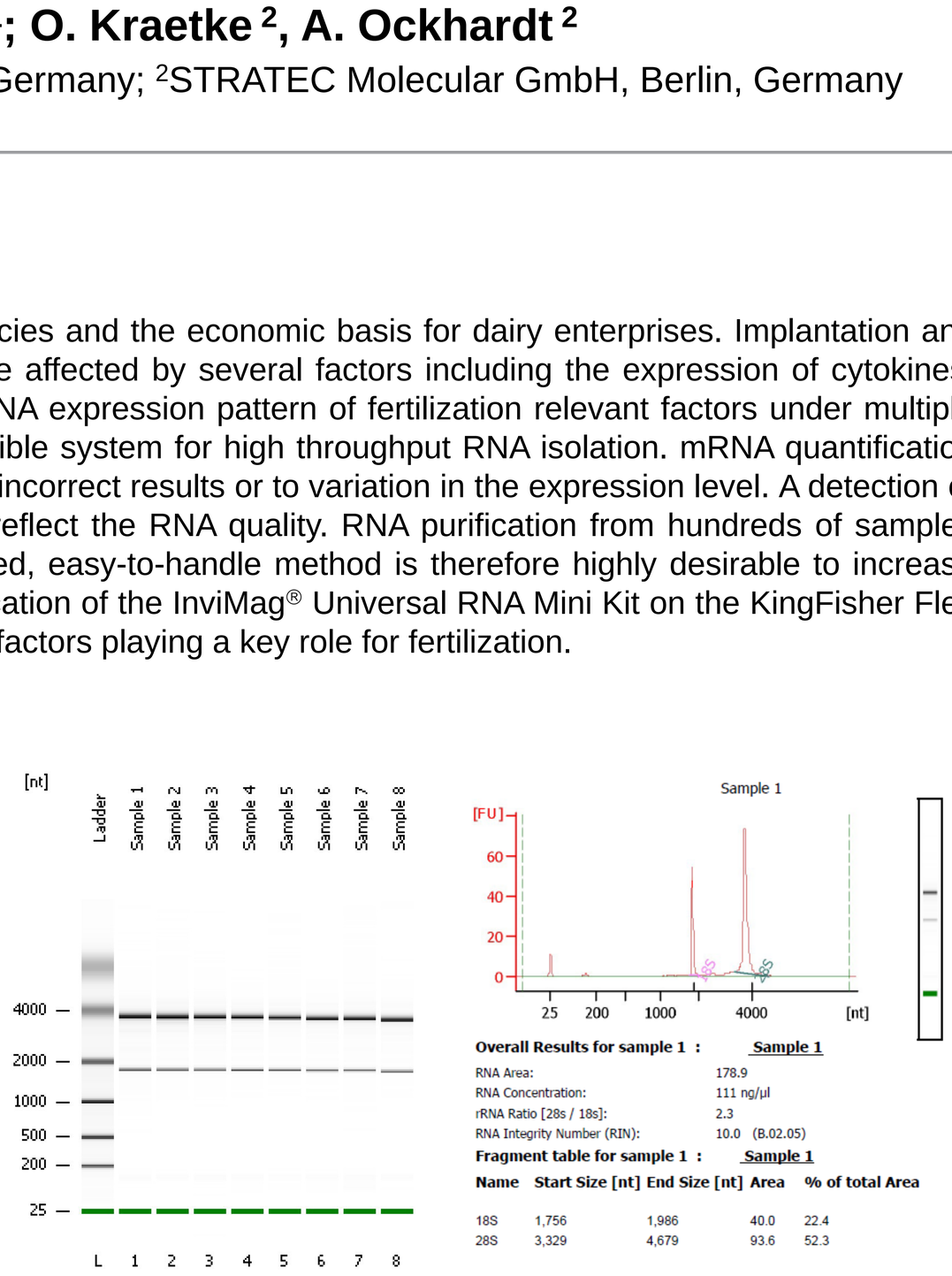

Fig. 1: A representative result for the RNA purity and integrity (RIN) is shown.

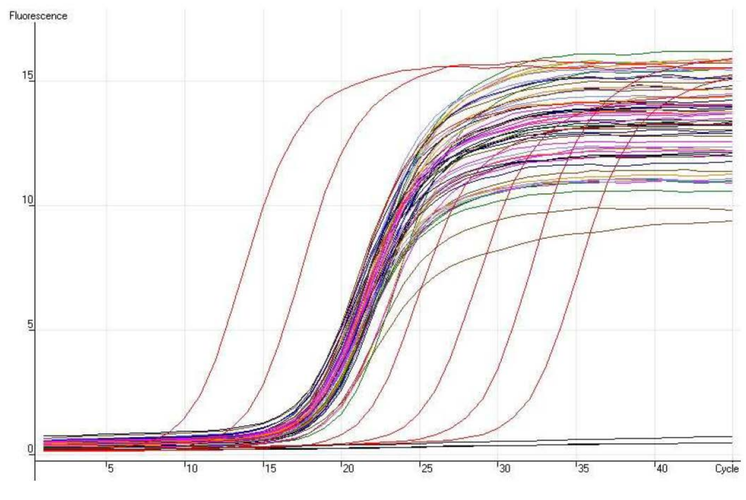

Fig. 2: Expression pattern of the house-keeping gene GAP-DH. An aliquot of each eluate was used for a real-time RT-PCR reaction for GAP-DH. All samples showed amplification signals.

SUMMARY

The use of the InviMag® Universal RNA Mini Kit on the KingFisher Flex 96 provides the following advantages:

a) high quality total RNA recovery without DNA digestion
- RIN factor in average of 9.9
- ratio A_{260}: A_{280} 2.05

b) fast isolation of RNA from 96 samples in approx. 60 min

c) convenient handling

d) error prevention for high throughput analysis
- all samples are processed on the same plate, at the same time and using standardized high reproducible procedures (automated system)

In conclusion the use of the InviMag® Universal RNA Mini Kit on the KingFisher Flex 96 for the analysis of the expression pattern of fertilization relevant factors underlines the RNA integrity, the efficiency, reproducibility and convenience of this tool for high throughput RNA isolation.

1) Reference: Odau S, Gabler C, Holder C, Einspanier R (2006) Differential expression of cyclooxygenase 1 and cyclooxygenase 2 in the bovine oviduct during the estrous cycle. J Endocrinol 191:263-274.