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# Follicular flushing in natural cycle IVF does not affect the luteal phase – a prospective controlled study

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**KEY MESSAGE**

Follicular flushing in natural cycle IVF did not affect luteal phase length or luteal phase progesterone and oestradiol concentrations. These results question the need for luteal phase supplementation in monofollicular IVF treatments.

A B S T R A C T

In contrast to multifollicular IVF, follicular flushing seems to increase the efficacy of monofollicular IVF treatments such as natural cycle IVF (NC-IVF). However, because follicular flushing causes loss of granulosa cells, it might negatively affect luteal phase length and endocrine function of the luteal body. A prospective cohort Phase II study was performed in 24 women undergoing NC-IVF. Women underwent a reference cycle with human chorionic gonadotrophin-induced ovulation without follicle aspiration and analysis of the length of the luteal phase and luteal concentrations of progesterone and oestradiol. In addition, they underwent a NC-IVF cycle which was performed identically but follicles were aspirated and flushed three times. The luteal phase was shorter in 29.2%, equal in 16.7% and longer in 50.0% of cases following flushing of the follicles. Overall, neither difference in luteal phase length was significant [median duration (interquartile range) in reference cycle: 13 (12; 14.5), flushing cycle: 14 (12.5; 14.5), median difference (95% CI): 0.5 (–0.5 to 1.5)] nor median progesterone and oestradiol concentrations. In conclusion, follicular flushing in NC-IVF affects neither the length of the luteal phase nor the luteal phase concentrations of progesterone and oestradiol, questioning the need for luteal phase supplementation.



## Introduction

Natural cycle IVF (NC-IVF) is being carried out at the request of patients (Højgaard et al., 2001) with increasing frequency in many countries (FIVNAT; Gordon et al., 2013;). The psychological distress for women (Haemmerli Keller et al., 2015), as well as the risks and costs (von Wolff et al., 2014) per treatment cycle, appear to be less compared with conventional IVF (cIVF) with gonadotrophin stimulation.

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However, these benefits only exist if the treatment cycles are optimized, i.e. the chance of success is maximized and the therapy is simplified. Egg collection is therefore of particular importance, because usually only one follicle can be aspirated. Follicular flushing is therefore often performed as this leads to an increased egg cell collection rate (Méndez Lozano et al., 2007; von Wolff et al., 2013) and the IVF success rate is consequently expected to increase. This is in contrast to cIVF, where no improvement in the egg cell collection rate was detected in normal responders with follicular flushing (Levy et al., 2012).

Although definitive proof of an increase in the pregnancy rate as a result of follicular flushing is still lacking for NC-IVF, based on the published studies to date and the little additional time required for the follicular flushing (Mok-Lin et al., 2013), follicular flushing is considered by several IVF centres (Méndez Lozano et al., 2007; von Wolff et al., 2013). However, it is completely unclear whether follicular flushing could also have a negative effect. It is possible that follicular flushing impairs the function of the corpus luteum and thus reduces the pregnancy rate.

As a temporary reduction in luteal progesterone levels was reported after follicle aspiration in normal cycles (Frydman et al., 1982), it is conceivable that additional follicular flushing possibly affects the formation of the corpus luteum even more because granulosa cells are flushed out of the follicle (Schneider et al., 2013). In order to reduce the risk of luteal phase insufficiency, luteal phase supplementation is usually applied (Pelinck et al., 2002). However, the benefits of such supplementation in NC-IVF are not clear, in contrast to cIVF where the benefit has been demonstrated (van der Linden et al., 2015).

Luteal phase supplementation increases the stress on women during the course of the monthly NC-IVF treatment cycles, because the vaginal progesterone administration may be accompanied by vaginal symptoms (Kleinstein et al, 2005, Simunic et al., 2007). To optimize the NC-IVF, i.e. not only to maximize the chance of success, but also to simplify the treatment cycles, luteal phase supplementation should only be carried out if its benefit has been demonstrated.

As a result of this, we conducted a prospective Phase II study with the aim of using the length of the luteal phase and the luteal concentrations of progesterone and 17β oestradiol to investigate whether aspiration, combined with repeated follicular flushing, affects the luteal phase and the endocrine function of the corpus luteum to gather additional evidence for or against luteal phase supplementation in NC-IVF.

## Materials and methods

### Study population and participants

A prospective cohort study was performed with 24 women undergoing NC-IVF in 2013 and 2014 in a university-based infertility centre.

Forty-nine women (age 18–40 years) with regular menstrual cycles (26–32 days), anti-Mullerian hormone concentrations >5 pmol/l, FSH concentrations <10 IU/l and without endometriosis > stage II rAFS were screened.

Forty-six were enrolled and 24 women completed the study (drop-out rate: 52%). Women underwent a reference cycle with human chorionic gonadotrophin (HCG)-induced ovulation without follicular aspiration, followed by analysis of the length of the luteal phase and concentrations of progesterone and oestradiol on day 2–3, 6–7 and 10–11 post-ovulation. In addition, a NC-IVF cycle was identically performed but follicles were aspirated and flushed three times (flushing cycle). As the order of the cycles was not strictly defined, women were also allowed to undergo the flushing cycle first. NC-IVF patients were monitored using ultrasound and analysis of LH and oestradiol concentrations. When the follicle diameter was expected to reach at least 16 mm and the oestradiol concentration was expected to be ≥800 pmol/l, 5000 IU of HCG (Pregnyl®, MSD Merck Sharp and Dohme GmbH, Lucerne, Switzerland) were administered 36 h before oocyte retrieval. Follicles were aspirated without anaesthesia and without analgesia using 19G single lumen needles (220 mmHg) as described elsewhere (von Wolff et al., 2013). After initial aspiration, follicles were flushed and aspirated three times each with 2–5 ml flushing medium with heparin (SynVitro® Flush, Origio, Berlin, Germany). The flushing volume was adapted according to the size of the follicle. Fertilization was achieved by standard intracytoplasmic sperm injection (ICSI).

Serum samples were collected in our infertility clinic and directly analysed for progesterone and oestradiol in the laboratory of the hospital by electrochemiluminescent immunoassay (ECLIA) on a COBAS 6000 (e601Modul) (Roche Diagnostics GmbH, Mannheim, Germany). The inter-assay coefficients of variation of these assays were less than 4%.

### Statistical analysis

It was expected that the flushing procedure would reduce the luteal phase length (primary outcome). We expected a shortening of the luteal phase in the flushing cycles in ≥80% of the participants. *A priori* sample size calculation determined that 22 subjects would be required to detect a shortening of the luteal phase length with 80% power ($P < 0.05$). Secondary outcomes were progesterone and oestradiol concentrations in the early, mid and late luteal phase in the reference and flushing cycles.

The length of the luteal phase of the reference versus flushing cycles was analysed using a paired Wilcoxon signed rank test with continuity correction. Because of multiple ties in the data, we used a normal approximation instead of the exact test.

Progesterone and oestradiol levels were analysed using paired Wilcoxon signed rank tests with continuity correction in the reference versus flushing cycle for each time point separately. No correction for multiple testing was done.

For the primary and secondary endpoints, we calculated the median of the differences and respective 95% non-parametric confidence interval (CI) from the test statistics which we present alongside the crude medians and interquartile ranges (IQRs). Our assessment of differences and the direction of differences are based on the median of differences, not the differences in the crude medians.

We used R 3.1.1 for all statistical analyses and for creating the figures (R Core Team, 2014).

### Ethical approval

Informed consent was obtained after the study procedures were explained to patients and all questions were answered. The study was approved by the Ethics Committee of the Canton Berne, Switzerland on 19 February 2013 (reference number KEK-BE 206/12) and registered with the German Clinical Trials Register (DRKS), DRKS00005248 on 28 August 2013.

## Results

Twenty-two women were excluded from the analysis (**Figure 1**). Eleven of those women became pregnant, requiring exclusion as the length



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