EFFECT OF NORETHISTERONE ACETATE* WITH OR WITHOUT ETHINYL OESTRADIOL ON THE SIALIC ACID CONCENTRATION AND SPERM RECEPTIVITY OF THE CERVICAL MUCUS

By

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ABSTRACT

The sialic acid concentration in the cervical mucus was studied during 9 ovulatory cycles in 5 subjects and in 5 other subjects before, during and after treatment with norethisterone acetate and ethinyl oestradiol in combination (Anovlar®) or norethisterone acetate alone. Sperm receptivity was also studied in the latter 5 subjects. In all cycles pregnanediol determinations and basal body temperature were used as an indication of ovulation. The following results were obtained:
1. The concentration of sialic acid decreases during the proliferative phase of the menstrual cycle and increases during the secretory phase.
2. In 19 of 25 cycles, cyclic changes in sialic acid concentration were found together with a positive sperm receptivity while in cycles with no change in sialic acid concentration no sperm receptivity was found.
3. During combination therapy (Anovlar®) no cyclic changes in sialic acid concentration, and no sperm receptivity were found.
4. During treatment with 1 mg of norethisterone acetate alone there was no sperm receptivity but in 2 of 5 subjects cyclic changes in sialic acid concentration were found.

* Norethindrone Acetate in the USA (17α-ethinyl-19-nortestosterone).
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It has repeatedly been claimed that an antifertility effect of a gestagen could be due to changes in the cervical mucus (CXM) \cite{London1967, Cohen1965, Rudel1965} in that it will become impenetrable or hostile to sperm. In the normal ovulatory CXM the sperm may survive for several days while the postovulatory CXM is impenetrable to sperm \cite{Lamar1939, Barton1946, Cohen1951}.

During the normal menstrual cycle the glandular cells of the cervix are subjected to fluctuations in hormonal levels in the blood, mainly oestrogens and progesterone. This is reflected in variations in quality and quantity of CXM. Several methods of evaluating the quality of CXM have been described, such as »Spinnbarkeit«, fern test, viscosity, tackiness and sperm propagation. Recently the nuclear magnetic resonance of the mucus has been shown to change characteristically during the menstrual cycle \cite{Odeblad1966}. Many tests are difficult to standardize and the evaluation may differ among investigators \cite{Platt1966}. Moreover, there are very few tests based on biochemical methods suitable for routine work \cite{Moghissi1966}.

Sialic acids have been shown to be part of the mucoproteins and are located in an end position \cite{Gottschalk1963, Dische1963, Sobrero1963}. They are the first moieties to be cleaved off the molecule during hydrolysis. There is substantial evidence that the CXM consists of long threadlike molecules of glycoprotein with cross-linkages occurring via the sialic residues of the glycoprotein \cite{Gibbons1966}.

The hormonal dependence of the sialic acid concentration in the mucus was shown by studying the mucification in the mouse vagina \cite{Carlborg1966}. Oestrogens were shown to decrease the content of sialic acid in the mucoproteins, whereas progesterone alone left it unchanged. However, an optimal combination of oestrogen and progesterone caused a marked incorporation of sialic acids in the mucoproteins. Characteristic variations were also recorded during the oestrous cycle, pregnancy and pseudopregnancy.

The purpose of this study was to evaluate the CXM before, during and after treatment with Anovlar\textsuperscript{®} or 1 mg norethisterone acetate (NEA) alone. Two methods were used; the determination of sialic acid concentration and sperm receptivity. The results were then correlated with the excretion patterns of pregnanediol and the basal body temperature, in order to assess whether ovulation had occurred.

**METHODS**

**Sialic acid determination**

The samples of CXM were dried in a 55\degree C water bath under nitrogen current using ethanol – acetone – ether as desiccants. After calculating the sample dry weight, sialic acid determinations were made using the Direct Ehrlich method \cite{Werner1952, Carlborg1966}. In pilot experiments the Thiobarbiturate method \cite{Warren1959}
was used and the same relative differences were recorded. The Direct Ehrlich method was chosen because of simplicity. The data are presented as µg sialic acid per mg dry CXM (sialic acid concentration).

**Sperm receptivity tests**

A modified version of the Miller-Kurzrok test (Kurzrok & Miller 1932) was first performed. The sperm used fulfilled the requirements given by McLeod & Gold (1956) with regard to concentration, volume, motility and the highest permissible per cent of abnormal forms. During the performance of the test, care was taken to completely surround the CXM to sample by sperm. This was important in order to prevent false negative results (Tampion & Gibbons 1962).

If the Miller-Kurzrok test was positive (sperms entering freely into the CXM), an attempt was made to determine the day of optimal sperm receptivity. Some CXM was sucked up into a capillary tube to about 15 mm, and fresh sperm was allowed to enter from one end. The distance at which the fastest sperm migrated in five minutes was taken as the end point. In Table 1, the day of maximum sperm receptivity is presented.

**Material**

A. The first experiment*, 5 normally menstruating women (age 23–32 years) were studied during 9 cycles. CXM was collected daily and was used solely for the determination of sialic acid. About day 10 and again at day 22, the urinary pregnanediol excretion was measured (Goldzieher & Nakamura 1962). A premenstrual increase in pregnanediol excretion and a rise in basal body temperature were considered suggestive of ovulation.

B. The subjects studied in the second part of the investigation were the same as those (except no. 6) in whom the levels of urinary gonadotrophins and pregnanediol were studied in response to treatment with Anovlar® or 1 mg NEA (McCormick et al. 1968). The experimental plan was as follows:

| No treatment / Anovlar® / No treatment / 1 mg NEA / No treatment |
|-----------------------------/-----------------------------/-----------------------------/-----------------------------/-----------------------------|
| 1st cycle                  / 2nd cycle                  / 3rd cycle                  / 4th cycle                  / 5th cycle                  |

The CXM was collected about ten times per menstrual cycle. No collection was made if bleeding occurred as this material was unsuitable for assay. In some cases, especially during the treatment periods, sufficient material could not be obtained for assay. Therefore, some determinations were made using material pooled from more than one day. The specimens were immediately stored in a refrigerator at −23°C.

**RESULTS**

A. In all nine ovulatory cycles, a midcycle fall in sialic acid concentration was recorded, the mean being on day 16. Day 16 was designated 0 and the days preceding were numbered −1, −2, etc. and the days following +1, +2.

* Performed by one of us (LC) at the Worcester Foundation for Experimental Biology. Shrewsbury, Mass., USA. Head: Dr. G. Pincus.
A compound curve was drawn using a 3-day moving mean (Fig. 1). The study covers day 7–22. Before and after this period it was difficult to obtain a suitable sample because of menstrual bleeding or insufficient CXM. From day –4 to +5 it was possible to calculate the standard error of the mean. From Fig. 1 it will be seen that a continuous decrease in sialic acid concentration occurred reaching the lowest value of 20–25 µg/mg dry weight of CXM on day 16, followed by a rapid increase.

The temperature curves obtained were all biphasic and the thermal shift on the average was simultaneous with the lowest sialic acid concentration.

B. The analysis of sialic acid concentration and maximum sperm receptivity will be presented together. For summary of events, see Table 1. Pregnanediol measurements in the latter part of the cycle were used as an index of ovulation.

In 14 cycles a postovulatory increase in sialic acid concentration was found while in 4 non-ovulatory cycles no change in the sialic acid concentration was noticed. Three cycles could not be studied because of insufficient material of CXM. In two cycles no post-ovulatory increase in sialic acid concentration was noticed. Cyclic changes in sialic acid concentration were found together with a positive sperm receptivity in 12 cycles. In 7 cycles absence of cyclic changes in sialic acid concentration was found together with a lack of sperm receptivity.

First cycle, untreated controls

All subjects had sialic acid concentration curves of the ovulatory type. The day of lowest sialic acid concentration coincided with that of maximum sperm receptivity. The pregnanediol measurements indicated ovulation in all subjects. Fig. 2 (subject SS) shows typical data for an untreated cycle.

Fig. 1.
Concentration of sialic acid in the cervical mucus. Compound curve of nine ovulatory cycles (mean values ± 1 s. e. m.).
Table 1.
Sialic acid concentration, sperm receptivity and pregnanediol excretion in 5 subjects treated with Anovlar® or 1 mg norethisterone acetate.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Untreated</th>
<th>Anovlar®</th>
<th>Untreated</th>
<th>1 mg Norethisterone Acetate</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS</td>
<td>S 13 R 13 P2</td>
<td>W N —</td>
<td>13 14 +</td>
<td>M N —</td>
<td>13 13 +</td>
</tr>
<tr>
<td>PC</td>
<td>15 13 +</td>
<td>M N +</td>
<td>W 17 +</td>
<td>M N +</td>
<td>21 12 +</td>
</tr>
<tr>
<td>SS</td>
<td>17 17 +</td>
<td>M N —</td>
<td>14 15 +</td>
<td>15 N +</td>
<td>11 11 +</td>
</tr>
<tr>
<td>LJ</td>
<td>13 13 +</td>
<td>M N —</td>
<td>13 N +</td>
<td>M N —</td>
<td>19 N +</td>
</tr>
<tr>
<td>IL</td>
<td>10 10 +</td>
<td>W N —</td>
<td>14 13 +</td>
<td>12 N —</td>
<td>M N +</td>
</tr>
</tbody>
</table>

S = day of midcycle minimum in sialic acid concentration if followed by an increase.
W = insufficient material available for analysis. M = monophasic or continuously decreasing values.
R = day of maximum sperm receptivity if present. N = no receptivity.
P2 = pregnanediol excretion. + indicates an increase in the latter part of the cycle to ovulatory values. — indicates a low excretion throughout the cycle.

Second cycle, Anovlar® treatment
The amount of CXM was very scanty throughout the cycle. Some samples necessitated pooling, resulting in an average of six samples per cycle. The determination of sialic acid concentration indicated a constant value of about 40 μg/mg dry CXM. No sperm receptivity was found in any sample. With the exception of one cycle no increase in pregnanediol was found.

Third cycle, first untreated after Anovlar®
Essentially all subjects resumed the normal ovulatory pattern of sialic acid concentration coinciding with the maximum sperm receptivity. One subject (LJ) demonstrated no sperm receptivity. All subjects showed an increase in pregnanediol excretion.

Fourth cycle, 1 mg NEA
In 2 cycles an increase in pregnanediol excretion was recorded, and in one of these cycles there was also a cyclical change in the sialic acid concentration. However, sperm receptivity was negative in all cycles. In Fig. 3 (subject LJ) the sialic acid concentration curve showed a continuous decrease without any premenstrual increase.
Concentration of sialic acids in the cervical mucus, pregnanediol excretion and basal body temperature in an ovulatory menstrual cycle (first cycle, subject SS).

_Fifth cycle, first untreated after 1 mg NEA_

All subjects but one resumed the normal ovulatory pattern of sialic acid concentration as well as positive sperm receptivity. All showed a premenstrual rise in pregnanediol excretion.

**DISCUSSION**

The possibility of ovulation occurring during treatment with combined contraceptive preparations has been demonstrated by Goldzieher et al. (1962). They used norethindrone 10 mg combined with mestranol 60 µg and showed that about 7 per cent of the treated patients had an increase in pregnanediol excretion in the latter part of the cycle. This finding was confirmed in this study and it is also shown that ovulation apparently can occur following treatment with 1 mg of norethisterone acetate alone. As these compounds are very
effective as antifertility agents may be suggested that they have some mode of action other than the inhibition of ovulation. The changes in sialic acid concentration and sperm receptivity of the cervical mucus which occur following treatment with these compounds may well be among the additional phenomena produced by these agents and responsible for their antifertility effect. Theoretically, therefore, a low dose of gestagen which has no ovulatory inhibitory effect may still be used as a contraceptive if it exerts an effect on the cervical mucus. One mg of norethisterone acetate rendered the cervical mucus impenetrable to sperm.

Cyclic changes in sialic acid concentration suggested ovulation. Thus, by taking repeated samples of cervical mucus in women, information may be obtained about the occurrence of ovulation.

Sialic acid determinations and sperm receptivity in samples of cervical mucus.
give valuable information in the evaluation of contraceptive compounds. It is suggested that more effort should be directed in obtaining agents which produce peripheral obstacles to sperm migration rather than inhibition of ovulation. Such compounds which may be administered in small amounts may cause less interference with homoeostasis and consequently may be better tolerated.

REFERENCES


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