

THE ACTION OF OXYTOCIN ON SPERM MOTILITY IN VITRO EXPERIMENTS WITH BULL SPERMATOZOA

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Summary: The effect of oxytocin on sperm motility of bull spermatozoa was investigated over a period of 2.5 hours. Measurements were carried out with a Lazymot apparatus, depending on the Laser-Doppler principle. In a concentration of 10 IU/ml, oxytocin was found to significantly increase the percentage of motile spermatozoa and sperm velocity compared with saline controls.

Key words: Oxytocin; Sperm motility; Laser-Doppler spectroscopy; Bull spermatozoa.

INTRODUCTION

Oxytocin, well-established as a female hormone, has recently been found present in males also. It has been detected in the testes of both animals and humans^(1, 3, 5, 7). Reports indicate that in the male it stimulates the muscles of the seminiferous tubules⁽⁸⁾ so promoting transport of the sperm. Therefore it seemed of interest to examine whether oxytocin also has a direct action on spermatozoa. Previous observations on bull sperm (Leipnitz) have indicated a qualitative increase in sperm motility. Thus, in the present study, a quantitative examination of the action of oxytocin on sperm motility has been made.

METHOD

The Lazymot apparatus was used to determine sperm motility, measurements depending on the Laser-Doppler principle. Deepfrozen bull-spermatozoa in pellet-form⁽²⁾ were thawed shortly before investigation, in Taumilch. This is a nutrition fluid consisting of: sterile homogenised milk (1.5% fat) 1000 g, aqua dest. 1000 g, glucose 21.50 g, sodium citrate 7.25 g, sodium hydrogen carbonate 2.36 g, citric acid 0.75 g, potassium chloride 0.20 g, glycerin 4.69 g. Oxytocin was added in equal volume to the individual samples so that the concentrations in the samples were 0.01, 0.1, 1.0 and 10.0 IU/ml. As control, an equal volume of saline was added to the sperm fluid. In each sample there was an average of 95 mill. sperm/ml (range 20-239 mill/ml).

Each of the samples and control (5 samples) was measured for 72 sec at 6 minute intervals over a period of 114 min (20 readings). Due to time required for preparing samples and adjusting the apparatus, the first reading was obtained 30 min after the addition of the test solutions. Each concentration of oxytocin and saline control was tested on 3 different sperm preparations. Temperature at which the samples were measured was 36 ± 0.01 °C. The following two parameters were estimated: percentage of motile spermatozoa and mean sperm velocity. Statistical evaluations were performed using the Tukey test after 2 factorial analyses of variance with 3 repeated measurements⁽⁴⁾.

RESULTS

Fig. 1 shows the values measured for percentage of motile spermatozoa (upper graph) and mean sperm velocity (lower graph) under the influence of oxytocin and saline. In the upper graph, the mean values of all test solutions are depicted. Oxytocin at a concentration of 10 IU/ml is represented by the top curve, whereas the curves of the other 3 concentrations tested are not distinguishable from that of saline. Because similar values were obtained for the mean sperm velocity, the lower graph simply compares the curves obtained for 10 IU/ml oxytocin with that of saline. As may be seen, the motility decreased continuously with time in all cases. In both motility parameters measured, the highest oxytocin concentration

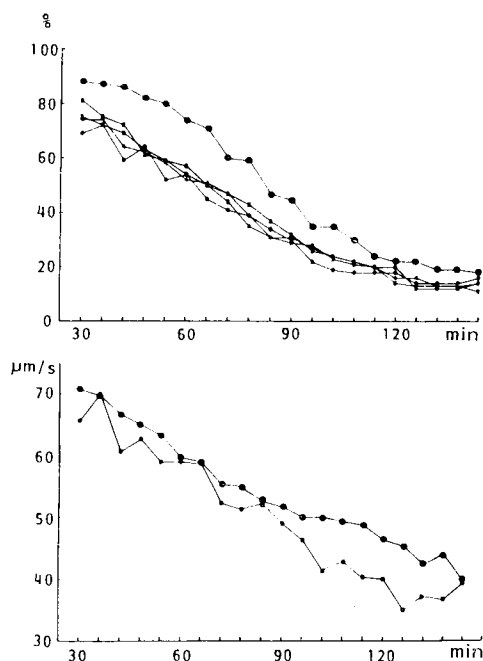


Fig. 1. - Action of oxytocin and saline on sperm motility. The upper graph shows percentage of motile spermatozoa, the lower graph mean sperm velocity over the time measured. In both graphs the upper curves represent the action of 10 IU oxytocin/ml. The action of the lower oxytocin concentrations tested is not distinguishable from that of saline. Therefore in the lower graph only the saline curve is shown for comparison. Each point represents the mean of three measurements.

(10 IU/ml) curve differed markedly from the saline curve.

To interpret the difference in action, the integral values measured over the period of 30 - 144 min. after addition of the test solution, were calculated for all oxytocin concentrations and for saline. These calculated values are illustrated in Fig. 2 in the form of columns. As seen, only the highest oxytocin concentration is markedly distinguishable from saline concerning percentage of motile spermatozoa. The difference is statistically significant ($\alpha=0.5$).

In a comparison of mean sperm velocity, differences are less marked but the action of the highest oxytocin concentration is still significantly different from that of saline ($\alpha=0.5$).

DISCUSSION

The action of the highest concentration of oxytocin tested has confirmed earlier qualitative findings by causing a quantitative increase in both motility parameters

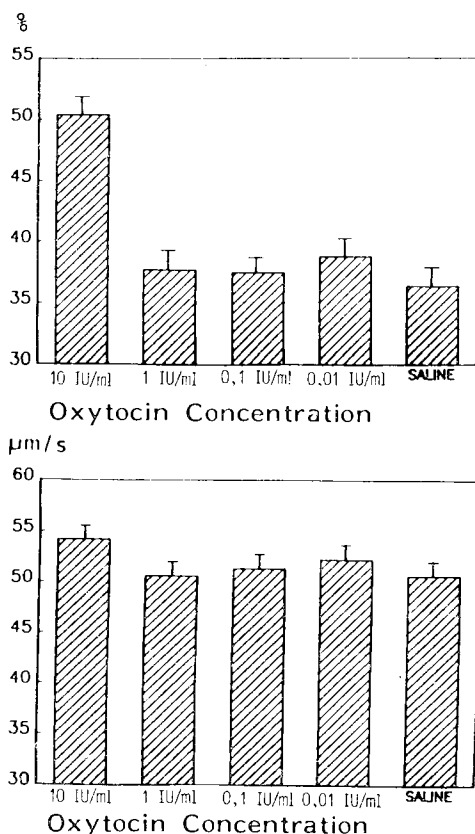


Fig. 2. - Action of oxytocin and saline on sperm motility. The upper graph shows percentage of motile spermatozoa, the lower mean sperm velocity; in both the integral values over the measured time period are expressed in the form of columns. Each column represents the mean value from three samples with standard deviation.

measured. In the present investigation, the sperm fluid was not separated from the spermatozoa as is usual when testing the action of pharmacological agents. However, earlier observations were also made on unwashed spermatozoa. According to the design of the present experiments, no information was obtained during the first 30 min. after addition of the test solution but possibly during this time the action was still more pronounced. However, the oxytocin action demonstrated is prolonged because, as can be seen in Fig. 1, after 2.5 hrs there is still an obvious difference between it and that of saline. It would be interesting to examine the oxytocin content of bull sperm and to investigate the pharmacological action of oxytocin using washed sperm. Since oxytocin has been shown to be present in the sperm of some animals^(1, 3, 5, 6, 7) and humans⁽³⁾, it may well play a physiological role in sperm motility.

Besides having an action on the motility of the ductus seminiferous⁽⁸⁾, a direct action on the sperm of the bull has now

been observed. Further examinations may show whether the motility of human sperm is also increased in this way.

ACKNOWLEDGEMENT

We thank Mr. F. Philipp from BTG Biotechnik GmbH München for kindly providing the apparatus Lazymot.

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INFECTIVE-HEMORRHAGIC COMPLICATIONS OF CESAREAN SECTION

A case review of 2220 subjects

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Summary: The Authors consider the hemorrhagic-infective complications associated with cesarean section. The review comprised 2200 subjects undergoing cesarean section from 1983 to 1987 in the Obstetrics and Gynecology Unit (Director: D. Dargent) of the University of Lyon (France). The incidence of cesarean sections was 14.9%, and endometritis was diagnosed in 17% of cases. The incidence of infective complications was 25% of cases and that of major hemorrhage 1%. The Authors conclude that the potential complications must be carefully considered whenever contemplating delivery by cesarean section.

Key words: caesarean section; complications.

INTRODUCTION

In spite of its widespread acceptance as a relatively easy procedure, cesarean section (CS) presents certain risks.

Recent reports (^{2, 6}) consider the different mortality and morbidity rates associated with cesarean section and with spontaneous or surgically induced vaginal delivery.

The most frequent peri-and post-operative complications are hemorrhages and infections, respectively. Inevitably, the quality and type of surgery will depend on the circumstances indicating surgical treatment.

Our extensive case review (2220 cases in 5 years) investigated various fetal and maternal conditions and permitted us to draw a number of conclusions as to the effective incidence of infective-hemorrhagic processes associated with cesarean section.

MATERIAL AND METHODS

From 1983 to 1987, 2220 cesarean sections were performed in the Gynecology and Obstetrics Unit (Director: D. Dargent) of the H. Herriot Hospital in Lyon, France. During the same period, 12,682 vaginal deliveries were performed.

Table 1 shows the maternal mortality. None of the pregnant women died directly as a result a cesarean section (two patients admitted to the Intensive Care Unit died as an indirect result

Table 1. - *Causes of maternal mortality (HEH, 1983-87).*

	Cesarean sections (2,220)	Vaginal deliveries (12,682)
Direct	0	1 Acute pregnancy-induced steatosis of the liver
Indirect	2 Aorta rupture Carotid insufficiency	0

Table 2. - *Obstetric hemorrhages (HEH, 1983-87).*

	Cesarean sections (2,220)	Vaginal deliveries (12,682)
Severe	24 (1%)	59 (0.46%)
Hemostatic hysterectomies	3	1
Transfusions	58 (2.6%)	195 (1.5%)

Table 3. - *Post-partum infections (HEH, 1983-87).*

	Cesarean sections (2,220)	Vaginal deliveries (12,682)
T > 38 °C	560 (25%)	543 (4.2%)
Endometritis	155 (7%)	328 (2.5%)
Serious infections	1/100	1/400
Surgery due to sepsis	4	3

of cesarean section). Hemorrhage as a complication is illustrated in Table 2, while infective complications are reported in Table 3.

DISCUSSION

Recently, maternal mortality related to cesarean section has decreased considerably.

The multicenter study carried out in 1986 by Boulanger (²), estimated maternal mortality in France at 1.66‰.

It is difficult to calculate the incidence of mortality as a direct consequence of CS; it probably ranges from 0.41‰ to 1.38‰.

North American studies, published according to thoroughly reliable criteria from 1976 onwards, report figures ranging from 0 to 59 per 100,000 operations (⁶).

Analysis of the cases considered definitely shows that the risk associated with CS is greater than that observed with vaginal delivery, though the relative risk is difficult to evaluate and quantify.

The most frequent peri-operative complications are hemorrhages.

In our Unit, only 1% of cases with hemorrhages received blood transfusions. This figure is much lower than the 10% reported by Boulanger (⁶), and clearly reflects the fact that we rarely prescribed blood transfusions, but preferred to rely on an procedures aimed at achieving perfect hemostasis, including hemostatic a-graffes in the hysterotomy area.

We agree with Pierre *et al.* (⁷) on the systematic utilization of oxytocic and other agents to stimulate uterine contraction after delivery and in the post-operative period. Severe hemorrhage (with blood loss exceeding 1 liter) was observed in 1% of our cases and is regarded as a relatively rare occurrence. Thorough study of coagulation mechanisms is required, as well as precise knowledge of the obstetric condition.

We performed three hemostatic hysterectomies (one case with placenta previa, one with placenta accreta, and one with silent rupture of the uterus). This operation is exceptional and should be avoided, not only because of the unfortunate "mutilating" effect it has, but also because of its high mortality rate: 3225 cases out of 100,000 cesarean hysterectomy were reported by Lehman *et al.* (⁵). Infection as a complication occurs much more frequently. According to various authors, the incidence varies from 15 to 40% of all cesarean sections. These values are much higher than the 3.5% frequency observed with vaginal delivery. In our study, a temperature greater than 38° C (excluding the day of surgery) was found in 25% of subjects.

In these cases, a systematic blood culture was obtained. Gibbs (³) estimates that 10-20% of patients with endometritis showed the presence of bacteremia. His figure closely resembles our own. Blood culture results are essential to specifically inhibit the growth of *S. aureus* cultures and prevent "metastatic" infections. Seven

percent of our cases were diagnosed as endometritis, which is similar to the incidence reported in various English language reports. Different bacteria are involved: in 80% of cases anaerobes are associated with aerobes, including *Escherichia coli* (30%) group B *Streptococci* (15%) *Klebsiella*, *Proteus mirabilis*, *Staphylococcus aureus* (5%) and group A *Streptococci* (less than 1%). Antibiotic treatment was administered. We prefer a combination of ampicillin and metronidazole, though Gibbs (³) believes that the clindamycin-gentamycin combination yields a better response (90-96%) initially and subsequently decreases the risks of infection.

In major infections we face the problem of re-operation, which proves necessary in cases of peritonitis. These types of peritonitis are very serious, and the outcome depends on how quickly re-operation is performed, on administration of antibiotics specific for anaerobes and on the possible use of hysterectomy in more severe cases (⁹).

The role of antibiotic prophylaxis remains controversial. Rayburn (⁸) reports that it decreases infective complications by 50%. In our study, we found that, even when used in short-course regimens (after clamping the umbilical cord in order to avoid contaminating the bacteriologic sample taken from the newborn), it cannot prevent most infections. Moreover, the possible development of endometritis would be more difficult to treat. Recently, single-dose cephalosporin prophylaxis has been proposed (⁴). Our experience with this regimen is too limited to permit us to draw any definite conclusions.

CONCLUSIONS

During recent years, cesarean section has modified the practice of obstetrics. Although we are far from the incidence reported in American countries (Brazil

Table 4. - Indications for cesarean section.

When woman is not in labor:	1,380 (62%)
- diseases during pregnancy	415
- dystocia or malpresentation	176
- previous cesarean sections	447
- concomitance of above factors	342
When woman is in labor:	840 (38%)
- abnormal course of labor	409
- fetal distress	255
- both combined	157
- others	19

26%), USA (25%) and Canada (24%), the figure in our unit was 14.9%. Table 4 summarises the indications for CS in our group of patients. Proper surgical procedures and strict observance of antiseptic norms considerably decrease hemorrhagic-infective complications. Maternal mortality as a complication of CS is very rare. Morbidity and mortality are much higher in CS than in vaginal delivery and this must be remembered when considering the indications for CS.

PRIMARY NON-HODGKIN LYMPHOMA OF THE VAGINA: CASE REPORT

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Summary: The Authors consider a rare case of non-Hodgkin primitive lymphoma of vagina, 1st E stage for Ann Arbor classification.

The treatment was primarily chemotherapy, because of the wide extension of the neoplasia and the elder patient age, and it was followed by a complete clinical response, still lasting after two years.

The results of treatments in literature are at least examined and discussed.

The female genital tract is primarily involved in malignant lymphomas with an incidence rate varying from 4 to 30%^(1, 2). Vaginal primary involvement is quite rare, with only 5 cases reported in Literature^(1, 2, 3, 4).

In our Institute we observed one case of primary non-Hodgkin lymphoma of the vagina.

CASE REPORT

M.G.M., aged 71, P4014, sought our attention on June 17th 1986, because of blood spotting from the external genitalia, during the past three months, with rectal tenesmus and stypsis. A phy-

sical examination was carried out and showed: external genitalia with senile dystrophy, the vagina with a stenosis of the lower two thirds, so as to make digital examination extremely difficult. The vaginal walls showed an induration which made it impossible to explore the internal genitalia (fig. 1). By rectal examination the mucosa appeared smooth, and the internal genitalia appeared in senile involution. Inguinal-crural lymphnodes were palpable, of parenchymal consistency, movable on external and deep layers. The pathological report showed massive corium involvement by atypical lymphoid cells, showing a non-Hodgkin lymphoma mildly differentiated (fig. 2). All studies of peripheral blood and a bone marrow biopsy were normal. Clinical and instrumental staging were negative for secondary localizations, so that the patient was classified IE stage (one single extranodal localization) according to the Ann Arbor classification.

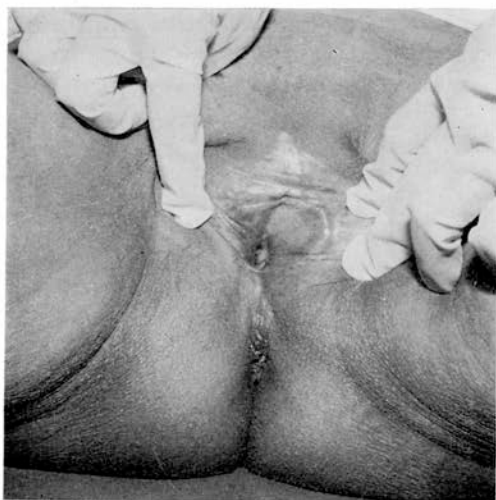


Fig. 1.

Because of the remarkable extension of the lesion (involving two thirds of the vagina), the age of the patient and the chemoresponsiveness of these neoplasias, treatment was given using polychemotherapeutical agents such CVP (Cyclophosphamide 500 mg/m² os 1st-5th day, Vinblastine 6 mg/m² e.v. 1st day, Prednisone 25 mg/m² os 1st-5th day; pause for 21 days) repeated for four cycles. At the end of the treatment a physical examination showed a complete regression

of the neoplastic involvement and a reestablishment of elasticity of the vaginal walls, so that we could easily introduce the vaginal valves (fig. 3). The patient is actually free of disease two years later, and her quality of life is good.

DISCUSSION

Non-Hodgkin lymphomas of intermediate grade of malignity, such as the case reported, may often present primary extranode localizations, especially in the stomach and encephalon. Histologically they may be represented by the follicular or the diffuse type, with small large cell patterns (centroblastic lymphomas). These are particularly aggressive course neoplasies (except the centroblastic type which more often appears at I-II stage), so they often appear from the beginning at stage IV. As to the staging of neoplasia, FIGO classification is insufficient, because it does not consider the secondary involvement of lymphnodes, which makes prognosis worse. Wherever the Ann Arbor classification is more suitable, because it considers nodal and extranodal involvement. According to that classification cases reported in Literature, including our case,

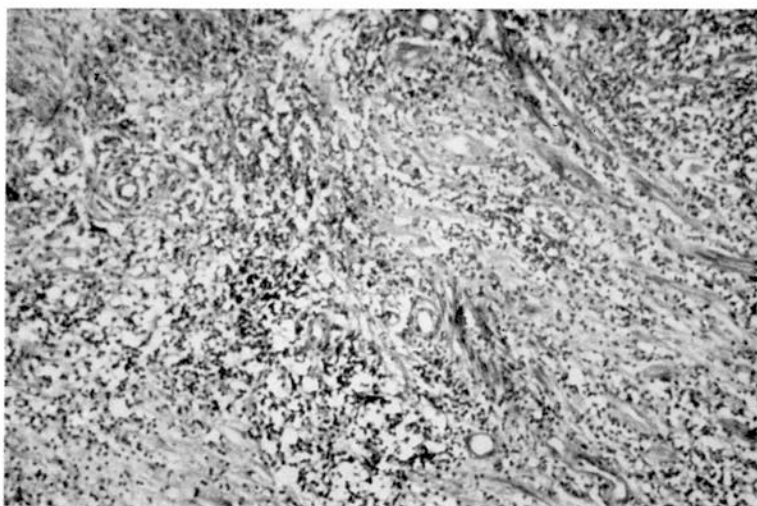


Fig. 2.

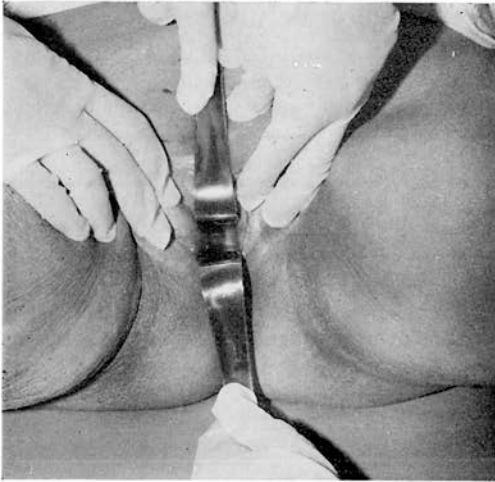


Fig. 3.

have been classified at IE stage, with good correlation to prognosis.

Chemotherapy is the primary treatment, giving a complete and persistent remission of disease; even a clinical reestablishment is possible, with a survival-rate of 50-70% at five years in stage IE of Ann Arbor.

Surgical therapy is suitable as a pre-chemotherapeutical reducing treatment, associated or not with Radiotherapy. Three of five cases reported in Literature underwent radical surgical management (one case of a Schauta-Amreich radical vaginal hysterectomy, one case of radical abdominal hysterectomy with colpectomy of the upper 3rd, followed by Radiotherapy, one case of anterior evisceration followed by radio- and chemotherapy), and two chemotherapy alone. Among the surgically treated cases there was a relapse in the only one which was not completed by chemo-radiotherapy, while the two cases treated by chemotherapy alone showed a favourable course.

In our case report the patient's age and the remarkable extent of the neoplasia

indicated primary chemotherapy, which determined complete clinical remission actually lasting for two years.

Though may be obtained good results by chemotherapy alone, it is necessary, in order to reach complete and lasting clinical remission, to associate radical surgery, when possible, or debulking surgery, according to the extension of the lesion and patient's health condition. For the same reasons surgery alone may not be considered as sufficient: in fact it is possible to have a relapse of disease, as in the case described in Literature, in which surgery was not completed by chemo- or radiotherapy.

In conclusion, non-Hodgkin lymphoma of the vagina, usually at intermediate malignancy (like all cases reported in Literature) has a relatively good prognosis, especially as it regards the centroblastic type, which is more often localized at a single site (stage IE of Ann Arbor). Chemotherapy is the elective treatment in these neoplasias, preceded if possible by surgery, at least debulking surgery, which allows the reaching of better responses to the medical treatment.

New perspectives seem to appear using systemic Interferon and rat monoclonal antibodies by intravenous administration⁽⁵⁾, with complete response ranging from 30 to 50%.

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