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In vitro Effect of Oxytocin on the Duration of Sperm Motility and Morphology

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Abstract: This study was designed to measure the effect of oxytocin on sperm morphology and prolongevity of motility of boar sperm kept at 18° C in diluent. Semen were taken from the same boar and diluted with citrate buffer. Diluted semen was containing $47x10^6$ Sperm mL^{-1}. Two mL^{-1} aliquots of diluted semen were placed into a series of test tubes. The tubes were added with $20\mu L^{-1}$ of saline containing oxytocin to give a final concentrationof 5 i.u. oxytocin,mL^{-1} were kept as test group while the tubes added just only with $20\mu L^{-1}$ saline were kept as control group. The tubes were kept at 18°C and at 0, 3rd, 19th, 26th, 42nd, 49th and 56 th h three samples from each group were taken out for motility and morphology measurements. Percentages of zero time motility were taken as 100%. The mean time taken for 15 and 50% decrease in total motility for control and test groups (Mean±SD) were 41.0±1.0 and 35..0±4.8; 25.6±5.0 and 29.0±9.8 respectively. The mean time taken for 6% and 50% decrease in forward motility in control and oxytocin treated group were 33.7±3.0 and 33.4±7.0; 12.4±3.3 and 12.5±3.4 respectively. Neither of the differences in mean times taken for decreases in motilities, were statistically significant. The differences in sperm morphology were not also significant ($p>0.05$). Sperm motility and morphology are important parameters for fertilizing ability of sperm. These results show that addition of 5 i.u. oyxtocin has no effect on the mean time taken for certain decreases sperm motility or sperm morphology. Therefore oxytocin did not increase fertilizing ability of sperm *in-vitro*.

Key words: Oxytocin, morphology, motility, sperm, boar

INTRODUCTION

The main aim of this study is to preserve the fertility potential of semen for few days above freezing. However though freezing of semen from cattle is a method of choice for storage and transport, but semen from some other species (boar and other wild spices including rabbit) requires short storage due to the difficulties in freezing or short term storage is required [1,3].

For this reason, 5 i.u. oxytocin was added to diluted semen samples at 18°C. Oxytocin is a neuropeptide which is released from posterior pituitary. It modulates mammalian sexual behaviour. In addition to affecting sexual behaviour, it has been also shown to increase cervical contractions, modulation of sperm production and transport [4]. It also effects secretion of sex steroids. An experiment in mice showed that intra-testicular injection of oxytocin increased basal testosterone level [5]. Due to the positive impact of oxytocin on the sperm production and the secretion of the sex steroids, it is negligible that the motility and the morphology of sperm cells in semen samples will be influenced by the addition of oxytocin.

The effect of adding 10 $iumL^{-1}$ oxytocin on bull sperm motility was investigated and it was reported that addition of oxytocin significantly increased the percentage of motile spermatozoa and sperm velocity [6]. In another experiment, male dogs were treated with 10 i.u. oxytocin and it has been reported that the total number of sperm in the ejaculate was increased, but there were no changes in all other ejaculate characteristics, such as progressive motility of sperm cells or percentage of morphologically normal spermatozoa [7]. In an *in-vivo* study, Holstein bulls were injected via the jugular vein with 50 i.u. oxytocin 10 minutes before each ejaculate for 10 weeks. Injection of oxytocin did not change the total number of spermatozoa harvested per collection day. Sperm motility and the percentage of spermatozoa with intact acrosome were also not changed by the injection of oxytocin [8]. In another experiment, 0.2, 2, and 20 i.u. oxytocin was added to bovine ejaculate (extended in egg-yolk citrate), the motility was not increased, but addition of 20i.u. of oxytocin to buffalo semen increased sperm survival [9,10]. In this study, 5 i.u. of $oxytocinmL^{-1}$ added to semen samples to measure its effect on sperm motility and morphology which are important parameters of fertility potential of semen.

MATERIALS AND METHODS

Collection of semen: The boar used in this experiment was 20 months old reared at a commercial farm. Before semen collection, a sow was introduced to the boar and the boar mounted the sow. As the penis extended, the operator hand grabbed to the tip and directed the ejaculate in to a funnel fitted with a filter paper to remove

jell fraction. The semen collected in the funnel and brought to laboratory in a thermos flask at 37°C.

Preparation of diluent and semen dilution: The diluent was prepared as follow:

- 60 g Fructose (Sigma, F2793)
- 3.7 g Tri sodium Citrate (BDH, 10242 4L)
- 0.5 g Streptomycin sulphate (Sigma, S0890)
- 0.312 g Penicillin G (500.000 units) (Sigma; P7794).

The above chemicals were made up to 1 litre with distilled water pH 7.3. The diluent was added to sperm (1:4 dilution). The diluted semen sample was placed in a water bath at 37°C and then a drop of semen placed on a makler counting chamber then a 0 time motility test was performed.

Preparation of oxytocin: Oxytocin was prepared by dissolving 1000 i.u. oxytocin in 10 mL^{-1} distilled water and aliquated to 1 mL^{-1} then frozen for future use as stock oxytocin. This stock oxytocin was diluted with isotonic salina to achieve a final concentration of 5i.u. oxytocinmL^{-1}.

Additon of oxytocin: Oxytocin was added either at the begging of the experiment (at 0 time) or at 19 hours of experiment.

Preparation of Nigrosin/Eosin/Giemsa stain: 10g Nigrosin and 0.7g eosin (sigma) were dissolved in a conical flask with 60 mL^{-1} distilled water. The flask was suspended in boiling water for 20 minutes. Dissolved nigrosin/eosin mixture was filtered through glass wool into a 100mL^{-1} measuring cylinder. Then 7.5 mL^{-1} of 50mM Glucose and 7.5 mL^{-1} of tartrate phosphate buffer (1 L TPB containing 7.10 g (50 mM) Di sodium hydrogen orthophosphate-anhydrous and 21,73 g (25m M) Potassium sodium tartrate) were added. The flask containing die was rinsed with this buffer and the total volume of die was brought up to 100 mL^{-1} by rinsing with tartrate buffer). Giemsa solution (Sigma, modified in buffered methanol solution, 0.4% w/v), pH 6.8, with stabilizers) was prepared by taking 4.5 mL^{-1} of giemsa solution with 3mL^{-1} of TPB and 3.25 mL^{-1} of distilled water on the day of use.

Staining: At 19, 26, 42, and 49 hours tree slides form each experimental group were prepared by mixing the samples with stain. According to sperm concentration, 4 drop of semen placed in a test tube by a transfer pipette and then 6-7 drop of nigrosin/eosin stain was placed in and left in a water bath at 37°C for a few minutes.

The smear was made by placing a drop of semen plus stain on a slide and smearing across the slide by another slide. For staining with giemsa, nigrosin/eosin stained smears were fixed for 7 minutes in TPB with 4% formaldehyde and then changed to fresh fixative for 3-5 minutes. The slides were rinsed in slow running water for 7-10 minutes then finally rinsed once with distilled water and immersed in giemsa working stain for 60 minutes. After one hour, the slides were dipped in following tap water and rinsed with distilled water then dried in air.

Measurement of sperm motility: The percentage of motile sperm was measured by using a makler counting chamber. The chamber was washed and dried then placed on the heating stage of a microscope to protect the sperm from the damage of cold. The specimen was mixed and a drop of semen was placed onto the centre of the lower disc and the cover glass was placed on then the number of forward moving, wigging and stopped sperm were counted within the squares of Makler at 200 magnification. For each sample, 200 sperms cell were counted. The percentages of total motile sperm and forward moving sperm were calculated as follows.
The type of o motility was classified as follows:

- Forward motility: Sperm moving rapidly in forward direction (FM)
- Wriggling: Sperm displaying flagellar movement without forward progression (W)
- Stopped: Sperm without any motion (S).
- Total number of sperm in mL^{-1} of semen (T) = FM+W+S
- Number of total motile sperm in one mL^{-1} of semen (TMS) = FM+W
- Percentage of total motile sperm per mL^{-1} of semen (%) = (TMS/T) x100
- Percentage of forward moving sperm (%)in one mL^{-1} of semen = (FM/T) x100

Measurement of time taken for percentage of decreases in total and forward motilities: Immediately after the dilution of semen motility tests were performed at,0,3, 19, 26, 42, 49 and 56 hours for control and test groups in triplicate. A figure showing the relation between percentage of decrease in motility and the time past was drawn. Form that figure percentages of decrease in total and forward motility, relative to time, were calculated.

Measurement of the percentage of morphologic abnormalities: 100 sperm on negrosin/esin stained slides were counted under a light microscope at 400

Table 1: The mean time (h) taken for 50% decrease in forward and total motility (h) when oxytocin added at the start of experiment

Type of motility	Replicate 1	Replicate 2	Replicate 3	Mean±SD
Forward motility				
Control	11.0	16.2	10.0	12.4±3.3
Oxytocin	10.2	16.4	11.0	12.5±3.4
Total motility				
Control	21.4	24.2	31.2	25.6±5.0
Oxytocin	24.4	22.0	40.2	29.0±9.8

Table 2: The time past for 6% decrease in forward and 15% decrease total motlity in control and oxytocin and control groups after the addition of oxytocin at 19h.

Type of motility	Replicate 1	Replicate 2	Replicate 3	Mean±SD
6% Decrease in forward motility				
Control	36.4	34.4	30.2	33.7±3.0
Oxytocin	41.4	27.4	31.4	33.4±7.0
15% Decrase in total motility				
Control	40.0	42.0	41.4	41.0±1.0
Oxytocin	34.7	30.4	40.0	35.0±4.8

magnification and the abnormalities were classified as detached head, bent tail, coiled tail, stoplasmic droplets and normal sperm. The slides were blind counted.

Statistical analysis: Data were analysed by using paired t-test.

RESULTS

Forward motility: When the oxytocin added at the beginning of experiment (at time 0), the mean time taken for 50% and 6% decreases in the percentages of forward moving sperm in control and oxytocin added samples were not statistically Significant ($p>0.05$) (Table 1 and 2).

Total motility: The results obtained show that addition of oxytocin either at the beginning or at 19 hours did not Prolonged ($p>0.05$) the mean time taken for 50% decrease in total and forward motilities (Table 1). The data displayed on Table 2, shows that the time past for 6% and 15% decreases in forward and total motilities are also not different ($p>0.05$). Morphological abnormalities: Table3 displays the changes in the mean percentage of morphological abnormalities after the addition of 5i.u.mL^{-1} oxytocin at 19 hours. The data indicates that oxytocin at 18°C has no effect on sperm morphology ($p>0.05$).

DISCUSSION

Addition of of oxytocin to semen samples or intra-uterin infusion of oxytocin before artificial insemination facilitates sperm transport in female reproductive tract and this may increase fertilization rate and consequent litter size [4]. During low fertility season (in summer period) addition of 4 i.ü. of axytocin into artificial insemination doses, just before insemination, has been reported to increase litter size This result attributed to the increase in uterine contractions because of oxytocin [11]. In another experiment, 5 i.u. oxytocin added to artificial insemination doses just before insemination and it has been reported that the sows inseminated with oxytocin added semen were superior to the controls by 6.3 per cent in pregnancy rate and by 56 born piglets to each 100 first inseminations and consequent results were significant. They reported that addition of oxytocin to semen samples prolonged motility more than 5 minutes and this resulted with better fertility results [12] while *in-vivo* injection of oxytocin to Holstein bulls [8] and to male dog [7] did not influenced sperm motility and morphology. It seems that increased litter size, in Peria and her collegue's experiment, is a result of increased sperm transport through female genital tract rather than a positive impact of oxytocin on sperm motility and morphology. In this study, 5 i.ü. of oxytocin was added to semen samples and no differences in sperm motility and morphology, between the control and the test groups, were found. However, the data presented here, indicate that addition of 5 i.ü. of oxytocin to boar semen do not affect fertility *in-vitro*.Bu in *in-vivo* conditions, addition of oxytocin to artificial insemination doses just before the insemination may have a positive impact on fertility. Because during natural mating the blood concentration of oxytocin in female is higher. Because , during natural mating, there is olfactory, visual auditory effect of male stimuli. During mating the male emits frothy saliva, produced from sub-maxillary salivary glands and urinates frequently.

The uterin and the saliva of mature boar contain large quantities of pheromones (e.g. 5á-androstenedione and 3á- androstenol). These pheromones increase oxytocin

Table 3: The changes in the mean percentage of morphological abnormalities of sperm treated either saline or oxytocin.

	Time (h)			
Type of abnormalities	19	26	42	49
Detached head				
Control	5.7 ±2.5	2.0 ±1.0	2.0 ±1.0	3.0 ±1.0
Oxytocin	5.3 ± 3.0	2.3 ±1.2	1.7 ±0.6	3.0 ±1.8
Lost acrosome				
Control	3.3 ±2.5	3.7 ±0.6	1.3 ±1.5	1.3 ±1.5
Test	5.3 ±1.2	3.3 ±2.5	1.7 ±1.5	1.0 ±1.0
Damaged acrosome				
Control	4.0 ±1.7	5.0±0.0	3.3±0.6	3.7±0.6
Oxytocin	4.0 ±1.7	5.3±1.5	6.0±0.0	4.0±0.0
Normal acrosome				
Control	92.7±4.0	91.3±0.5	95.3±2.0	95.0±2.0
Test	93.7±2.9	91.3±1.2	89.0±4.4	95.0±1.0
Bent tail				
Control	1.0±1.7	0.0±0.6	0.0	0.3±0.6
Test	1.0±1.7	1.3±2.3	0.0	1.0±1.0
Cytoplasmic droplets				
Control	7.7±3.2	7.7±4.0	5.7±0.6	8.0±3.5
Oxytocin	7.0±2.6	7.7±2.9	7.7±4.0	7.3±3.5

secretion within 8 minutes. In an experiment, 5á-androstenedione sprayed for 2 sec. in front of the sow's snout and that caused 2-to 10 fold increase in oxytocin secretion within 8 minutes. During natural mating a 40-fold increase in peripheral oxytocin concentration was observed [11]. Artificialy inseminated animal lacks educate male stimuli. Therfore, addition of axytocin to artificial insemination doses, just before insemination, is a good practical method to increase conception rate and fertility.

Increase in fertility, due to the addition of oxytocin, is a result of increase in sperm transport within the female genital tract rather than improved sperm motility and morphology.

The same thing may apply for other farm animal such as cows and ewes. Oxytocin can increase fertility of artificially inseminated cows and ewes when the insemination doses are added with oxytocin just before the insemination [9,10,13].

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