
Eficácia da abertura da zona pelúcida através do uso do laser em embriões humanos criopreservados: um estudo prospectivo, randomizado e controlado.

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Abstract

Purpose: It has been suggested, but not reliably documented in the literature that the process of embryo cryopreservation may affect the physicochemical characteristics of the zona pellucida (ZP) causing its hardening and apparently impairing blastocyst hatching. The objective of this study was to evaluate the efficacy of one laser-zona thinning (L-ZT) microdissection applied to thawed embryos transfers (ETS) compared to thawed ETS not submitted to L-ZT.

Methods: A total of 135 patients who were submitted to an ICSI-program and had their excess embryos frozen and thawed by the slow method were divided, in a prospective and randomized manner (2:1), into two groups at the time of receiving their thawed embryos: Group I (90) patients whose thawed embryos transferred were all submitted to L-ZT; Group II (45) patients whose thawed embryos transferred were not submitted to L-ZT (control group). In Group I, L-ZT was performed using a non-contact laser with a length of 1.48 μm (Fertilaser) with 1-2 irradiations of 10 ms applied to the ZP of each embryo to thin 60 to 80% of the ZP (length of the laser ZD thinning = 30 μm). Embryo transfer was performed soon after the measurement of ZP thickness using an eyepiece with a μm scale, and the L-ZT procedure. Data were analyzed by the Mann Whitney test.

Results: The age of group I patients (32.4 ± 4.7) did not differ (p = 0.3) from that of group II patients (32.9 ± 4.5). The number of cleaved embryos transferred was similar (p = 0.6) for the two groups (group I = 2.2 ± 1.0; group II = 2.1 ± 1.0). The embryo survival rate (50% intact blastomeres) after thawing did not differ significantly (p = 0.6) between group I (83.6% ± 14.5) and group II (81.4% ± 15.8). ZP thickness of group I embryos (17.3 ± 2.8 μm) did not differ (p = 0.4) from that of group II embryos (17.1 ± 2.4 μm). There were no significant differences between groups I and II with respect to the implantation rates (%) in the natural (group I = 11.7%; group II = 12.5%; p = 1) or substitutive (group I = 26.5%; group II = 20%) cycle. The clinical pregnancy rate per transfer of group I (26.6%) did not differ significantly (p = 0.5) from that of group II (20%).

Conclusions: Pregnancy and implantation rates were not different between the group submitted to L-ZT and the control group. In the present study, the use of L-ZT was not effective for frozen-thawed embryos. However, this conclusion is limited to a situation in which length of the laser zona thinning was 30 μm.

Key words: zona pellucida thinning, microdissection, cryopreservation, laser

Introduction

The zona pellucida (ZP) is an extracellular matrix that surrounds the oocyte and is known to have a variety of functions. Before fertilization, the ZP presents a species-specific sperm barrier and immediately after fertilization it may act by protecting against leukocyte infiltration and infection by bacterial or fungal
agents and by protecting the fragile oocytes and embryos against physical damage from the environment. Also, the ZP function to prevent the separation of blastomeres from cleaving embryos and to maintain the blastomeric arrangement to ensure successful subsequent development (Cohen et al., 1990). On the other hand, the poor implantation rate after the transfer of apparently normal looking embryos is one of the unsolved problems incurred in IVF. The process of oocyte and embryo cryopreservation may affect the physicochemical characteristics of the ZP. Carroll et al. (1990) observed that frozen-thawed mouse oocytes presented a reduced fertilization rate (48.8%) compared to non-cryopreserved controls (97%). The exposure of mouse oocytes to dimethylsulfoxide prior to cryopreservation can lead to a premature discharge of cortical granule contents and thereby to zona hardening (Schalkoff et al., 1989). In addition, Check et al. (1996) reported an increased implantation and pregnancy rate for patients whose frozen-thawed embryos were submitted to ZP microdissection.

On the other hand, holes in the ZP may cause loss of blastomeres, or loss of the whole embryo during contractions of the reproductive tract (Nichols & Gardner, 1989). It also deprives the embryo of its protective coat, which protects against any detrimental factors in the female reproductive tract. Previous studies using videocytomorphology of IVF embryos prior to transfer have shown that variability of zona thickness within individual embryos is one of the most significant morphological predictive factors of implantation (Cohen et al., 1989; Palmstierna et al., 1998). Gabrielsen et al. (2001) observed significantly better clinical pregnancy when ZP thickness variation was used as the selection criterion in cases where all embryos had an asynchrony in development or a high embryo score (i.e., were of poorer quality) by classical evaluation (odds ratio = 2.5). These data support the development of a technique such as zona thinning, whereby the variability of ZP is enhanced.

In zona thinning, the zona is just made thinner over a certain area without a hole or a slit being created and assisted zona thinning in both humans and in animal models has been previously reported to have a positive influence on hatching and implantation rates (Khalil et al., 1992; Antinori et al., 1996). Rinke et al. (1996) showed that a 1.48 mm diode laser system is safe and efficient for microdissection of the ZP of mouse oocytes without impairment of subsequent embryo development. The laser-assisted microdissection of the ZP can be applied with high precision, with a consequent reduction of the risk of causing damage to the embryo.

The objective of this study was to evaluate in a prospective and randomized manner the efficacy of laser-zona thinning (L-ZT) applied to thawed embryos (ETs) compared to thawed ETs not submitted to L-ZT.

Material and Methods

Patients

A total of 135 patients who were submitted to an ICSI program between January and December 2001 and had their excess embryos frozen and thawed by the slow method were divided in a prospective and randomized manner (by drawing lots, using a randomization table previously elaborated for the study) into two groups (2 experimental: 1 control) at the time of receiving their thawed embryos. Group I: (90) patients whose thawed embryos transferred were all submitted to L-ZT and group II: (45) patients whose thawed embryos transferred were not submitted to L-ZT (control group).

Ovarian stimulation protocol and ICSI procedure

All women underwent controlled ovarian and follicular stimulation after pituitary down-regulation as previously described (Franco Jr et al., 2001). Ovarian stimulation before the ICSI procedure consisted of blockade of the 2nd phase with nafarelin acetate at the dose of 400 μg/day (Synarel, Serad). After 14 days of treatment with the analogue and the establishment of blockade, administration of recombinant FSH was started at a fixed dose of 150-300 IU for a period of 7 days. On the eighth day of ovarian stimulation, we started monitoring follicular development only by vaginal ultrasound and the doses of FSH were adapted to the ovarian response. When at least 3 follicles measuring ≥ 17 mm in diameter were observed, hCG was administrated at the dose of 5000-10000 IU.

For sperm separation from seminal fluid a discontinuous gradient of Sperm-Prep 100 TM was used (IVF Science Scandinavia, Sweden) applied to the 40% and 90% fractions. The ICSI procedure was performed by the method of Svanlander et al. (1995). Fresh embryos were routinely transferred on day 2. All excess embryos were cryopreserved on day 2 regardless of morphological quality.

Slow freezing method

For the freezing process we used the Freeze-kit 1 (IVF Science Scandinavia, Sweden), consisting of the following solutions: PBS, 1.5 M propanediol (PROH) and 1.5 M PROH + 0.1 M sucrose. The solutions were then distributed on Nunc culture plates and allowed to stabilize at room temperature for 15 minutes. Cryopreservation was performed with a cryotank CL-863 apparatus. Excess embryos were first washed in PBS solution, transferred to 1.5 M PROH for 10 min, and then transferred to 1.5 M PROH solution with sucrose and immediately placed in straws. The straws were transferred to a cryochamber stabilized at a temperature of 24°C and temperature was then reduced at the rate of 2°C per minute until -6°C, when manual seeding was performed. The temperature was then reduced by 0.3°C per minute until -50°C, followed by a slow fall to -150°C, after which the straws were transferred to liquid nitrogen.

For the thawing process, the Thaw-kit 1 of IVF Science Scandinavia (Sweden) was used. The kit consists of four solutions: 1.0 M PROH plus 0.2 M sucrose, 0.5 M PROH plus 0.2 M sucrose, 0.2 M sucrose, and PBS. The solutions were stabilized at room temperature for at least 15 minutes and the straws were stabilized at room temperature for 30 s. The embryos were successively added to the four solutions (1.0 M PROH/0.2 M sucrose, 5 min; 0.5 M PROH/0.2 M sucrose, 5 min; 0.2 M sucrose, 10 min; PBS, 5 min). The embryos were then transferred to PBS stabilized at 37°C in the presence of 5% CO2 for 5 min, and finally incubated in G1.2 culture medium (IVF Science Scandinavia, Sweden) for 24 hours (Mauri et al.,

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Before transfer, the selected embryos were positioned and assessed for variation of zona thickness by measuring zona at four points (9, 12, 3 and 6 o'clock) using an eyepiece with a \( \mu \)m digital scale. The variability of ZP thickness was determined for each embryo in both groups (Cohen et al., 1989).

Transfer was performed after determination of embryo cleavage, which was considered to be present when the division of at least one of the blastomeres was observed after 24 hours of culture (Van der Elst et al., 1997).

**Replacement cycle and pregnancy**

Two hormonal schemes were used for the transfer of thawed embryos, i.e. the natural cycle and a substitutive cycle. In the natural cycle, follicular development was monitored by serial vaginal ultrasonography starting on the 10th day of the cycle. A 10000 IU dose of hCG was administered when the follicle presented a diameter \( \geq 17 \) mm, and thawing was routinely performed on the fourth day after hCG (day of hCG injection = day 1) and embryo transfer on the fifth day after hCG.

In the substitutive cycle, estradiol valerate (Postov, Wyeth, São Paulo, Brazil) was administered from the first to the 14th day of the cycle at a daily dose of 6 mg. Progesterone (Utrogestan, Basins International, France) was also introduced on the 14th day at the dose of 400 mg/day by the vaginal route, as long as endometrial thickness was \( \geq 6 \) mm (16). Thawing was performed on the fourth day of progesterone treatment and transfer on the fifth day.

The pregnancy test was performed on the 14th day after treatment and clinical pregnancy was confirmed during the sixth week by the presence of a gestational sac and an embryo with a heart beat. Data were analyzed statistically by the Whitney and Fisher tests.

**Laser-assisted microdissection of the zona pellucida**

ZP microdissection was performed by a 60 to 80% reduction of ZP thickness at a single site with the use of one to two 10 ns irradiations with a 1.48 mm wavelength diode laser (Fertilaser-MTM, Medical Technologies Montreux, Lausanne, Switzerland) operated through an objective coupled to an inverted Eclipse TE 300 microscope (length of the laser zona thinning = 30 \( \mu \)m). The thawed embryos that cleaved after 24 hours of culture in the laboratory were submitted to the process of ZP microdissection. After the procedure the embryos were always transferred to fresh culture medium to avoid the possibility of the products derived from the action of the laser on the organic components of the ZP. Cleaved embryo transfer was performed soon after ZP microdissection, i.e., 24 hours after the thawing process (Baruffi et al., 2000).

**Results**

A total of 135 cycles of post-thawing embryo transfer were performed in the present study: group I (laser thinning = 90 cycles) and group II (control = 45 cycles).

In the randomization process, only the patients in the first or second thawing cycle referring to the previous ICSI series participated in the process of drawing lots. In group I, 94.4% of the patients were analyzed in the 1st thawing cycle and 5.55% in the 2nd, and in group II 97.7% of the patients were analyzed in the 1st cycle and 2.3% in the 2nd (p = 0.66).

The 50% survival of blastomeres after thawing did not differ significantly (p = 0.6) between group I (83.6 \( \pm \) 14.5%) and group II (81.4 \( \pm \) 15.8%). Percent embryo cleavage after 24 hours in culture did not differ significantly (p = 0.48) between group I (81.1 \( \pm \) 27.7%) and group II (75.4 \( \pm \) 26%).

**Table I: Clinical and Laboratory data of the studied groups during fresh cycles.**

<table>
<thead>
<tr>
<th></th>
<th>Group I (L-ZT)</th>
<th>Group II (Not L-ZT)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>90</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>32.3 ( \pm ) 4.7</td>
<td>32.9 ( \pm ) 4.5</td>
<td>0.36</td>
</tr>
<tr>
<td>Number of collected oocytes</td>
<td>15.6 ( \pm ) 6.3</td>
<td>13.9 ( \pm ) 6.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Number of oocytes MII</td>
<td>12.6 ( \pm ) 4.9</td>
<td>11.9 ( \pm ) 5</td>
<td>0.41</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>70.4% ( \pm ) 16</td>
<td>74.7% ( \pm ) 14</td>
<td>0.19</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>2.6 ( \pm ) 0.7</td>
<td>2.6 ( \pm ) 0.8</td>
<td>0.69</td>
</tr>
</tbody>
</table>

ZP thickness of group I embryos (17.3 \( \pm \) 2.8 mm) did not differ (p = 0.4) from that of group II embryos (17.1 \( \pm \) 2.4 mm). The percent variation in ZP thickness in group I (13.6%) did not differ significantly (0.59) from group II (13%).

Table I presents the clinical and laboratory data for the patients of group I and group II in the fresh cycles when their excess embryos were frozen. The parameters analyzed: age, number of oocytes retrieved, oocytes in metaphase II, percent fertilization after ICSI, and number of embryos transferred did not differ significantly between groups.

Table II lists the clinical and laboratory data for the patients of groups I and II in the thawing cycles. There were no significant differences between groups I and II with respect to the following parameters: age, mean number of frozen embryos, mean number of thawed embryos, number of cleaved embryos transferred, implantation, clinical pregnancy and abortion rates.

**Table II: Clinical and laboratory details of the studied groups during the thawing cycles.**

<table>
<thead>
<tr>
<th></th>
<th>Group I (L-ZT)</th>
<th>Group II (Not L-ZT)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>90</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>32.4 ( \pm ) 4.7</td>
<td>32.9 ( \pm ) 4.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Frozen embryos</td>
<td>6.2 ( \pm ) 4.1</td>
<td>6.4 ( \pm ) 3.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Thawed embryos (total)</td>
<td>3.3 ( \pm ) 1.4</td>
<td>3.5 ( \pm ) 1.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Cleaved embryos transferred</td>
<td>2.2 ( \pm ) 1.0</td>
<td>2.1 ( \pm ) 1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>26.5%</td>
<td>20%</td>
<td>0.5</td>
</tr>
<tr>
<td>Abortion rate</td>
<td>20.8</td>
<td>22.2</td>
<td>1</td>
</tr>
</tbody>
</table>

A total of 90 embryo transfers were performed in group I, 60 in a natural cycle and 30 in a substitutive cycle, and a total of 45 embryo transfers, 23 in a natural cycle and 22 in a substitutive cycle, were performed in group II. There was no significant difference (p = 0.09) between groups I and II with respect to number of transfers in a natural or substitutive cycle. Tables III and IV show the rates of embryo implantation (IR) obtained for groups I and II with the natural (group I: IR = 11.7% and
group II: IR = 12.5%) or substitutive (group I: IR = 26.7% and group II: 20%) ovarian scheme. There were no significant differences between groups I and II with respect to the implantation rates in the natural (p=1) or substitutive (p=0.51) cycle.

Table III - Number of embryos implanted or not in a natural ovarian cycle in groups I and II

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Group I (L-ZT)</th>
<th>Group II (Not L-ZT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implanted</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Not implanted</td>
<td>113</td>
<td>42</td>
</tr>
<tr>
<td>P=1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table IV - Number of embryos implanted or not in a substitutive ovarian cycle in groups I and II

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Group I (L-ZT)</th>
<th>Group II (Not L-ZT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implanted</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Not implanted</td>
<td>47</td>
<td>40</td>
</tr>
<tr>
<td>P=0.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

There is evidence in the literature that culture conditions or the cryopreservation process itself may affect the physicochemical characteristics of the ZP and consequently the natural process of blastocyst hatching (Schalkoff et al., 1989; Carrol et al., 1990; Vincent et al., 1999). Tucker et al. (1991) observed that the use of ZP microdissection in thawed embryos increases the implantation rate (16%) compared to the control group (9%). Check et al. (1996), in a paired study, observed that the patients submitted to assisted hatching of frozen-thawed embryos obtained a significantly higher clinical pregnancy rate (30.4%) than that of the control group (15.2%). In 1999, Cohen et al. observed the efficiency of assisted hatching with acid tyrode solution in thawed embryos of patients submitted to a program of egg donation. The patients who received embryos submitted to assisted hatching (n = 49) presented higher implantation rates (23% versus 6.0%) and clinical pregnancy rates (45.8% versus 10.2%) than the group with no assisted hatching (n = 48). The authors also suggested that cryopreserved embryos should be submitted to the process of assisted hatching before transfer.

Despite these favorable laboratory data, there is no definition in the literature about whether ZP dissection is necessary in thawed embryos, nor is there information about the ideal technique to be used for this procedure.

On the other hand, while it seems that embryo holes in the ZP have an increased potential for earlier and more likely hatching, complete perforation of the ZP does impose certain drawbacks. Holes in the ZP may cause loss of blastomeres or loss of the whole embryo during contractions of the reproductive tract or embryo transfer; they also deprive the embryo of its protective coat, which protects against any detrimental factors in the female reproductive tract (Nichols & Gardner, 1989).

From an experimental viewpoint, Khalifa et al. (1992) observed that ZP thinning acts optimally to promote both early and complete hatching in the mouse. In a mouse model, they compared embryonic development after zona drilling, and cruciate thinning of the zona intended to simulate the natural thinning of the ZP. Cruciate thinning of the zona appears less detrimental than zona drilling. Both techniques significantly increased the rate of hatching, but zona drilling did not guarantee complete hatching. In addition, Cohen et al. (1990) have suggested that complete perforation of the ZP may have adverse effects on subsequent embryonic development.

In 1996, Antinori et al. reported that laser-assisted ZP microdissection by thinning applied to embryos from patients with repeated implantation failure resulted in higher implantation rates (12.2% versus 7.3%) and pregnancy rates (42.7% versus 23.1%) compared to a control group. The cited investigators also stated that the ZP was thinned for a 20 μm length to produce a 50% reduction of its thickness, making it very likely that the inner layer of the ZP was involved in the thinning process.

From a theoretical viewpoint, the advantages of performing ZP microdissection by thinning may be related to the philosophy of embryo protection by partial conservation of ZP integrity. In this way, it would be possible to avoid the aggression that may occur in the reproductive tract (embryo infection with bacteria, fungi etc.) or those directly linked to the increased pressure during the process of embryo transfer (herniation and loss of blastomeres). In addition, the rate of "in vitro hatching" was significantly higher in laser-thinned blastocysts compared with control embryos (Blake et al., 2001).

The major disadvantage may reside in the fact that the process does not fully break the so-called inner membrane of the ZP, a situation that may theoretically reduce the efficiency of the process aiming at facilitating the hatching process.

However, there are very few studies in the literature separately analyzing the thickness and/or consistency of the inner and/or outer membranes of the ZP. One of the reasons for this could be that imaging the architecture of the zona glycoproteins using transmission electron microscopy or scanning electron microscopy is technically challenging because their highly hydrated state complicates the process of fixation with conventional agents and mechanical stress introduced during freezing and drying creates artefacts by altering alignment of zona filaments. There are no precise reports about the thickness of the inner layer of the ZP in human embryos or oocytes. However, Keefe et al. (1997) observed by a recent modification of the polarized light microscope (pol-scope/non-destructive method to identify macromolecular organization) that the ZP of all hamster oocytes studied appeared to be subdivided into two birefringent layers separated by an anisotropic layer. The thickness of the three layers (inner, isotropic, and outer) was measured randomly and was found to be 6.10 ± 1.6, 1.87 ± 0.59 and 4.96 ± 0.80 μm, respectively (mean±SD). The thinning technique used in the present study produced a loss of ZP thickness of 60% to 80%, possibly damaging the inner layer of the ZP in a significant manner, since the inner layer theoretically corresponds to about half the total thickness of the ZP.

It is hard to define the best length of laser-assisted ZP microdissection. Mantoudis et al. (2001), in a non-randomized study, observed that clinical pregnancy rates arising from quarter laser-assisted hatching (L-AH) in comparison with...
pontal L-AH (single hole created without reaching the inner membrane) and total L-AH (single hole completely through the zona pelúcida). In 2001, Blake et al. assessed the efficacy and hatching characteristics of in-vitro cultured human embryos subjected to laser ZP thinning. Zona thinning was performed on 110 embryos using a non-contact 1.48 μm diode laser and the hatching rate in vitro was compared with 42 control embryos. A maximum of six ablations were made successively around the zona to achieve a depth of 50-80% of the zona thickness for a total length of approximately 80 μm. Scanning electron microscopy was performed on embryos entrapped during hatching to identify the site of hatching. The rate of hatching was significantly higher in laser thinning blastocysts compared with control embryos (68 versus 33%, p < 0.01). These results suggest that laser zona thinning is effective (in vitro hatching) and may provide significant advantages over conventional assisted hatching techniques, which create holes. However, no clinical studies evaluating the efficiency of ZP thinning over a greater extension (80 μm) in a prospective and randomized form are currently available. On the other hand, the choice of a hormonal scheme (natural or substitutive cycle) for thawed embryo transfer depended only on whether or not the patients had shown natural ovulation in a previous cycle. The influence of this variable on the final data was removed by randomization and was also confirmed by analysis of the results since there were no significant differences in the number of natural and substitutive cycles in either group or in the rates of implantation of embryos submitted or not to L-ZT (Tables III and IV). In addition, the clinical pregnancy rate per transfer of L-ZT patients (26.6%) did not differ significantly (p = 0.5) from that of the control group (20%). In the present study, the use of L-ZT was not effective for frozen-thawed embryos. However, this conclusion is limited to a situation where length of the laser zona thinning was 30 μm.

**Resumo**

Tem sido sugerido na literatura mas não está sendo documentado, que o processo de criopreservação de embrião pode afetar as características físico-químicas da zona pelúcida (ZP), causando dificuldades no hatching natural pelo blastocisto. Objetivo: o objetivo deste estudo foi avaliar a eficiência do laser no afinamento da zona pelúcida (L-ZT) através da microdissecação aplicada nos embriões congelados a serem transferidos, comparando com os embriões que não foram submetidos ao L-ZT.

Material e métodos: Um total de 135 pacientes que foram submetidos ao tratamento de fertilização in vitro – ICSI e possuíam embriões excessivos congelados pelo método lento foram incluídas neste estudo divididas em dois grupos de forma prospectiva e randomizada (2:1), no momento de receber os embriões descongelados: Grupo I: (90) pacientes as quais os embriões transferidos foram submetidos ao L-ZT; Grupo II (45) paciente cujo os embriões transferidos não foram submetidos ao L-ZT; grupo controle. Nos 1 o afinamento do zona pelúcida foi feito através de laser sem contato com comprimento de onda de 1.48 μm (Fertilaser). Um total de 1-2 irradiações de 10ms foram aplicadas na zona pelúcida de cada embrião a fim de aferir 60 a 80% da zona pelúcida (comprimento total do afinamento por laser = 30μm). A transferência dos embriões foi realizada logo após a medição da ZP e do seu afinamento. Os dados foram analisados pelo teste Mann Whitney. Resultados: A idade do grupo I (32 ± 4.7) não foi diferente (p=0.3) das do grupo II (32.9 ± 4.5). O número de embriões transferidos também foi similar (p=0.6) para os dois grupos (grupo I: 2.2 ± 1.0; grupo II: 2.1±1.0). A taxa de sobrevida de 50% dos blastômeros após o descongelamento não diferiu significativamente (p=0.6) entre o grupo I e o grupo II (grupo I =83.6 ± 14.5, grupo II =81.4 ± 15.8%). A espessura da ZP nos embriões do grupo I (17.3 ± 2.8 μm) não foi significativamente diferente (p=0.4) do grupo II (17.1 ± 2.4 μm). As taxas de implantação dos grupos I e II no ciclo natural foi grupo I (11.7%) e grupo II (12.5%) (p=1). No ciclo substitutivo foi grupo I: 26.7% e grupo II (20%) (p=0.51). A taxa de gravidez por transferência do grupo I foi (26.6%) e do grupo II (20%), tendo assim (p=0.5). Conclusão: As taxas de implantação e gravidez não foram diferentes entre os grupos: controle e o que foi submetido ao L-ZT. No presente estudo, o uso de L-ZT não foi efetivo para embriões congelados. Entretanto, essa conclusão é limitada para a situação em que o comprimento utilizado para o afinamento da zona era igual 30μm.

**References**


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