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BIOCHEMICAL PREGNANCIES AND THEIR MANAGEMENT

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SUMMARY

There is little information in the literature about biochemical pregnancies (BP). However, BP (a pregnancy that regresses before imaging with ultrasound) is a significant problem in IVF and recurrent miscarriage clinics. The incidence of between 13-22% of pregnancies may be confounded as today's sensitive pregnancy tests may detect endometrial, pituitary, or phantom hCG. A false positive result may also follow extraneous hCG administered in an ART cycle. Hence, the author has suggested a rising hCG level at two consecutive tests as a definition and that one raised hCG level should be known as a raised isolated hCG level. The etiology remains unclear. Embryonic aneuploidy, thinned endometrium, sperm defects, and defective angiogenesis have been suggested. Additionally, several biochemical pregnancies are early ectopic pregnancies that fail to develop further.

We see a subsequent live birth rate of 53% in untreated patients with two or more biochemical pregnancies. However, our team treats recurrent BP's as recurrent pregnancy loss (RPL) as suggested by ESHRE. However, treatment to prevent further BPs is empiric, with no evidence in the literature. The author uses hCG supplementation to enhance implantation. 61 of 87 patients (70%) with >2 biochemical pregnancies and 12 out of 14 patients (86%) with >4 biochemical pregnancies delivered with hCG supplementation. These figures compare favorably to the 53% live birth rate with no treatment, but power analysis shows that 228 patients would be required to show statistical significance. The author has used IVIg on 20 patients with >5 biochemical pregnancies, with 50% terminated as live births. However, the results may be confounded as the previous biochemical pregnancies may have been early ectopic pregnancies, and the subsequent pregnancy intra-uterine.

If a biochemical pregnancy becomes persistent and hCG levels fail to fall, methotrexate may be required, as in early ectopic pregnancies.

Keywords: Biochemical pregnancy (BP), hCG levels, in vitro fertilization (IVF), recurrent pregnancy loss (RPL), ectopic pregnancy, implantation enhancement, methotrexate treatment

Biochemical Pregnancy

The most common definition of a biochemical pregnancy (BP) is a positive βhCG test with no pregnancy on ultrasound. The most recent terminology is from the European Society of Human Reproduction and Embryology (ESHRE) in 2015.[1] The classification is based on previous definitions. If there is a decreasing βhCG level and no localization of the pregnancy on ultrasound, if performed, the pregnancy is known as a non-visualized pregnancy.[2] If no ultrasound has been performed, the pregnancy loss has been called a "biochemical pregnancy."[3] If the pregnancy resolves spontaneously after expectant management, it is known as a resolved pregnancy of unknown location after expectant management.[4]

However, in in vitro fertilization (IVF) programs, low levels of βhCG may be diagnosed and interpreted as a biochemical pregnancy. Consequently, past definitions include βhCG levels of 10-1000 IU and a rising level.[5, 6] An alternative nomenclature suggests raised isolated hCG levels and biochemical or non-visualized pregnancy if the hCG level rises without extraneous hCG administration.

It is questionable whether BPs should be recognized as pregnancies, early miscarriages, or implantation failures. The American Society of Reproductive Medicine (ASRM) distinguishes BPs from clinical pregnancies and does not recognize BPs as miscarriages, as raised isolated hCG levels may peak and rapidly fall, and there may be no delay in the onset of the next menstrual period. Additionally, as BPs cannot be localized, every biochemical pregnancy is a pregnancy of unknown location (PUL). PULs may be early ectopic pregnancies. The ESHRE recognizes BPs as miscarriages, partly based on Kolte et al's[2] work that each non-visualized pregnancy loss reduces the chance of a subsequent live birth by 10% (RR, 0.90, CI 0.83; 0.97), similar to the risk conferred by each additional clinical miscarriage. The author runs a dedicated clinic for women with recurrent pregnancy losses. In this clinic, there are many women with BPs and recurrent BPs. Our experience is similar to Kolte's[2] experience. Hence, the author does classify BPs as early miscarriages if there is a rising hCG level.

Raised Isolated hCG Levels

The mRNA for hCG has been detected in 8-cell embryos. hCG from 7 days after ovulation[7] and can be used clinically from 9 days after the LH surge. A positive hCG after 12 days is usually indicative of pregnancy. However, present tests are so sensitive that phantom, endometrial, or pituitary hCG can be detected. A low positive hCG does not invariably mean that trophoblastic hCG is present. Additionally, some tests use animal antibodies raised to hCG. If the patient harbors anti-animal antibodies after exposure to the same animal used in the test, there may be a false positive result. If hCG is used for ovulation induction, it may still be present after 12 days. Van Der Weier et al.[8] showed low amounts of hCG as a contaminant in hMG, and Kol[9] showed hCG as present in Corifollitrophin α. Intra- and inter-laboratory variation may also lead to false positive results. These low levels of hCG are raised isolated hCGs, not biochemical pregnancies.

Incidence

The prevalence of biochemical pregnancies has been reported to vary between 13-22% in fertile patients.[10,11] Isolated elevated hCG levels have been reported in 4% of Liu et al.'s series.[12] In the infertile population, the incidence has been reported to be 14-18%, which is not higher than in the fertile population.[13,14] A higher incidence has been reported in IVF patients (22-31%) compared to the general infertile population.[15,16] However, the incidence remains stable across all age groups and does not increase with age.[17]

Causes of Biochemical Pregnancies

The cause of biochemical pregnancies may depend on the embryo or the mother.

Embryo Causes:

hCG is essential for human implantation. The hCG produced at the start of pregnancy is mainly the hyperglycosylated form hCG-H.[18,19] hCG-H is autocrine in nature, created by the cytotrophoblast to drive invasion of the syncytiotrophoblast. According to Sasaki et al.,[20] only 8 of 36 biochemical pregnancies produced >40% hCG-H on the day of implantation, compared to 100% of pregnancies terminating at term. Alternatively, if implantation is delayed, a slow rise in hCG may indicate abnormal embryonic development, which may have occurred after implantation due to chromosomal or other embryonic factors.[21]

As recurrent implantation failures and miscarriages are often due to embryonic aneuploidy, it has been assumed that biochemical pregnancies may be due to a genetic aberration. Troncoso et al.22 reported a case-control study in which 62 patients underwent PGT, and their BP rates were compared to 62 patients undergoing embryo transfer on day 3 or 62 patients on day six after ovum pickup. The incidence of BPs was approximately 25% in all three groups. Hence, embryonic chromosomal aberrations were not the cause of BPs in most patients.

Maternal Causes:

Endometrial thickness has been reported to impact biochemical pregnancies. In Dickey et al.'s report,[23] BPs were found in 21.9% (7 of 32) of pregnancies if the endometrial thickness was less than 9 mm on the day of hCG administration in women undergoing ovulation induction, but none of 49 pregnancies when the endometrial lining was more significant than 9 mm. Hence, a thin endometrium may not allow proper invasion by the trophoblast and inappropriate placentation. Additionally, hCG secretion by the invading trophoblast may be negatively modulated by endothelin-1 (ET-1) or PG F2α found in the endometrium.[24] Oxidative stress can also enhance hCG levels while not allowing necrosis and apoptosis of the trophoblastic epithelium.[25]

Implications of Biochemical Pregnancies

The occurrence of a biochemical pregnancy is psychologically distressful for both partners. There is joy in achieving a pregnancy after prolonged infertility, only to have that happiness dashed by pregnancy loss. Hence, the stress associated with biochemical pregnancies has led to patients leaving IVF programs.[26]

The occurrence of a BP is a negative predictor for subsequent pregnancy outcomes, as BPs have higher recurrent BP and miscarriage rates.[27,28] In cases of exclusively recurrent biochemical pregnancies, the risk of ectopic pregnancy has been reported to be 27%.[29] However, 6% to 20% of women with sporadic BP have an ectopic pregnancy.[30]

Management

BPs may be non-viable or present with persistent raised hCG levels. In some cases, methotrexate (MTX) may be required to induce trophoblast regression. In ectopic pregnancy, MTX is associated with a 67-94% success rate. Side effects such as stomatitis, gastrointestinal distress, dizziness, neutropenia, reversible alopecia, abdominal pain, and vaginal bleeding or spotting may occur. After recurrent biochemical pregnancies, there is insufficient information from the literature to formulate guidelines for management. Below are some suggestions based on the author's experience, which are not evidence-based.

If there is one isolated BP, the author believes there is little need for active treatment. If there are two consecutive BPs, there is still little need for active treatment. However, ESHRE regards two BPs as two pregnancy losses and, therefore, can be assumed to support treatment to prevent recurrence. If there are three or more BPs, the author treats the patients as if there were three or more miscarriages. Our database contains the details of 87 patients with two or more BPs who did not receive active treatment in the index pregnancy. There were 61 subsequent live births (70%), not significantly different from 22/41 (54%) in the control group.

Power analysis shows that 410 patients are required to show statistical significance.

Specific Medications (Author's Experience):

There is little information on various drugs used to improve the live birth rate. As stated above, hCG-H accounts for 90% of the total hCG in the first two to three weeks of pregnancy when invasive trophoblast activity is highest.[18,19] Hence, a luteal dose of hCG is often administered in IVF practice to enhance implantation. Theoretically, hCG-H may prevent pregnancy failure at the time of implantation. However, hCG-H is patented and not commercially available; therefore, generally, commercially available hCG can be used instead. hCG prevents further miscarriages in recurrent miscarriage.[31] The author has used hCG supplementation in 34 patients with three or more BPs. 26 subsequent pregnancies terminated as live births (76%). However, the numbers are too small to determine if this 76% live birth rate significantly differs from the 61% seen in the control group (11 live births in 18 pregnancies). The author has used intravenous immunoglobulin in patients with five or more BPs. Ten live births were achieved in 20 pregnancies (50%).

CONCLUSIONS

Much more data is necessary on biochemical pregnancies. Databases must be combined to increase the number of patients available for assessment. One possible source of "big data" is the Society for Assisted Reproductive Technology (SART), but BPs should be reported as BPs and not early pregnancy losses. Detailed histological studies need to be performed on failed pregnancies. It is essential to understand if hCG-H levels are deficient and, if so, if hCG-H may prevent BPs.

REFERENCES

1. ESHRE Guideline Group on RPL, Atik RB, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelen W, Peramo B, Quenby S, Vermeulen N, Goddijn M. ESHRE guideline: recurrent pregnancy loss. Available from: http://www.eshre.eu/Guidelines-and-Legal/Guidelines.
2. Kolte AM, van Oppenraaij RH, Quenby S, Farquharson RG, Stephenson M, Goddijn M, Christiansen OB; ESHRE Special Interest Group Early Pregnancy. Non-visualized pregnancy losses are prognostically important for unexplained recurrent miscarriage. *Hum Reprod*. 2014; 29: 931-937.
3. Farquharson RG, Stephenson MD, eds. *Early Pregnancy*. New York: Cambridge University Press; 2010.
4. Barnhart K, van Mello NM, Bourne T, Kirk E, Van CB, Bottomley C, et al. Pregnancy of unknown location: a consensus statement of nomenclature, definitions, and outcome. *Fertil Steril*. 2011; 95: 857-866.
5. Carp HJ, Toder V, Mashiach S, Rabinovici J. Effect of paternal leukocyte immunization on implantation after biochemical pregnancies and repeated failure of embryo transfer. *Am J Reprod Immunol*. 1994; 31:112-115.
6. De Neubourg D, Gerris J, Mangelschots K, Van Royen E, Vercruyssen M, Elseviers M. Single top quality embryo transfer as a model for prediction of early pregnancy outcome. *Hum Reprod*. 2004; 19: 1476-1479.
7. Lopata A, Hay DL. The potential of early human embryos to form blastocysts, hatch from their zona and secrete HCG in culture. *Hum Reprod*. 1989; 4(8 Suppl): 87-94.
8. Van de Weijer BH, Mulders JW, Bos ES, Verhaert PD, van den Hooven HW. Compositional analyses of a human menopausal gonadotrophin preparation extracted from urine (menotropin). Identification of some of its major impurities. *Reprod Biomed Online*. 2003; 7: 547-557.
9. Kol S. False positive blood hCG test following Corifollitropin alfa injection. *Hum Reprod*. 2018; 33: 177.
10. Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. *Fertil Steril*. 1996; 65: 503-509.
11. Coulam CB, Chapman C, Rinehart JS. What is a preclinical pregnancy loss? *J Assist Reprod Genet*. 1998; 15: 184-187.
12. Liu HC, Jones HW Jr, Rosenwaks Z. The efficiency of human reproduction after in vitro fertilization and embryo transfer. *Fertil Steril*. 1988; 49: 649-653.
13. Salumets A, Suikkari AM, Makinen S, Karro H, Roos A, Tuuri T. Frozen embryo transfers: implications of clinical and embryological factors on the pregnancy outcome. *Hum Reprod*. 2006; 21: 2368-2374.
14. Zeadna A, Son WY, Moon JH, Dahan MH. A comparison of biochemical pregnancy rates between women who underwent IVF and fertile controls who conceived spontaneously. *Hum Reprod*. 2015; 30: 783-788.
15. Bjercke S, Tanbo T, Dale PO, Mørkrid L, Abyholm T. Human chorionic gonadotrophin concentrations in early pregnancy after in-vitro fertilization. *Hum Reprod*. 1999; 14: 1642-1646.

16. Hourvitz A, Lerner-Geva L, Elizur SE, et al. Role of embryo quality in predicting early pregnancy loss following assisted reproductive technology. *Reprod Biomed Online*. 2006; 13: 504-509.
17. Dahan MH, Zeadna A, Dahan D, Son WY, Steiner N. The biochemical pregnancy loss rate remains stable up irrespective of age and differs in pattern from clinical miscarriages. *Gynecol Endocrinol*. 2021; 37: 61-64.
18. Cole LA. hCG and hyperglycosylated hCG in the establishment and evolution of hemochorial placentation. *J Reprod Immunol*. 2009; 82: 112-118.
19. Evans J. Hyperglycosylated hCG: a unique human implantation and invasion factor. *Am J Reprod Immunol*. 2016; 75: 333-340.
20. Sasaki Y, Ladner DG, Cole LA. Hyperglycosylated human chorionic gonadotropin and the source of pregnancy failures. *Fertil Steril*. 2008; 89: 1781-1786.
21. Liu HC, Rosenwaks Z. Early pregnancy wastage in IVF (in vitro fertilization) patients. *J In Vitro Fert Embryo Transf*. 1991; 8: 65-72.
22. Troncoso C, Bosch E, Rubio C, Remohí J, Simón C, Pellicer A. The origin of biochemical pregnancies: lessons learned from preimplantation genetic diagnosis. *Fertil Steril*. 2003; 79: 449-450.
23. Dickey RP, Olar TT, Taylor SN, Curole DN, Harrigill K. Relationship of biochemical pregnancy to pre-ovulatory endometrial thickness and pattern in patients undergoing ovulation induction. *Hum Reprod*. 1993; 8: 327-330.
24. Sunder S, Lenton EA. Endocrinology of the peri-implantation period. *Baillieres Best Pract Res Clin Obstet Gynaecol*. 2000; 14: 789-800.
25. Jauniauz E, Poston L, Burton GJ. Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. *Hum Reprod Updat*. 2006; 12: 747-755.
26. Pearson KR, Hauser R, Cramer DW, Missmer SA. Point of failure as a predictor of in vitro fertilization treatment discontinuation. *Fertil Steril*. 2009; 91(4 Suppl): 1483-1485.
27. Yang R, Yang S, Li R, Chen X, Wang H, Ma C, Liu P, Qiao J. Biochemical pregnancy and spontaneous abortion in first IVF cycles are negative predictors for subsequent cycles: an over 10,000 cases cohort study. *Arch Gynecol Obstet*. 2015; 292: 453-458.
28. Tarín JJ, Pascual E, Gómez R, García-Pérez MA, Cano A. Predictors of live birth in women with a history of biochemical pregnancies after assisted reproduction treatment. *Eur J Obstet Gynecol Reprod Biol*. 2020; 248: 198-203.
29. Christiansen OB. Biochemical pregnancies—shall they count in the recurrent miscarriage diagnosis? *J Reprod Immunol*. 2011; 90: 155.
30. Kirk E, Bottomley C, Bourne T. Diagnosing ectopic pregnancy and current concepts in the management of pregnancy of unknown location. *Hum Reprod Update*. 2014; 20: 250-261.
31. Morley LC, Simpson N, Tang T. Human chorionic gonadotrophin (hCG) for preventing miscarriage. *Cochrane Database Syst Rev*. 2013; (1)

SUPERCHARGED MECHANICAL STROMAL-CELL TRANSFER (MEST)

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SUMMARY

PRP and fat-derived stromal cell applications are the most commonly used regenerative medicine methods. PRP has a broad spectrum of indications. Due to their advantages, mechanical methods have recently become very popular in fat-derived stromal cell applications. Combining these two methods has produced more successful results, providing reassurance about the effectiveness of the MEST method. This combination combines two products obtained separately before they are administered to the patient. In this study, fat tissue and blood samples obtained from eight volunteers were mixed with PPP, a new idea not previously reported in the literature, and stromal cells were obtained mechanically with sharp blades (adinizing). Later, the obtained PRP was added to the final product and became "supercharged." The results were tested by the dual fluoroscopy method for cell number and viability, and the results obtained were analyzed statistically. By adding the plasma to the oil before stromal cells were obtained and cutting with sharp blades by mechanical separation, twice the volume and 4.7 times more cells were obtained compared with that obtained in the saline group ($P < 0.001$). We believe that the reason for this is the "binding" effect of the proteins in the plasma. This approach provided a higher cell count using PPP, a "waste product," and increased potential efficiency by adding PRP. However, the clinical results of this innovative method should be evaluated with advanced clinical studies. (Plast Reconstr Surg Glob Open 2021; 9: e3552; doi: 10.1097/GOX.0000000000003552; Published online May 10, 2021)

Keywords: PRP, fat-derived stromal cells, regenerative medicine, MEST method, PPP, cell viability, mechanical separation

INTRODUCTION

In many medical disciplines, regenerative medicine has recently been a fast-growing and popular trend. Using fat-derived stromal cells and blood-derived platelet-rich plasma (PRP) is one of the

most common applications.[1] Stromal cells are obtained mechanically rather than enzymatically, not only because of legal restrictions but also because such procedures are more accessible and are capable of obtaining more cells efficiently and economically.[2] Obtaining stromal cells from adipose tissue by enzymatic method has been described elsewhere in detail.[3] To date, many devices have been applied in different ways. However, consensus has yet to be reached on the definition of the final product or even the preparation protocols in mechanical ways.[4] Copcu and Oztan, in their study published in 2020 on using sharp-knife systems, obtained a high number of stromal cells mechanically without creating blunt-force pressure.[2] The name they gave to the procedure of cutting fat tissue with a sharp knife was “adinizing” and represents the first time indication-based protocols were established for the final product, its desired physical structure (solid, liquid, emulsified), and the required number of cells. Unlike enzymatic methods, they suggested that the term total stromal-cell (TOST) should be applied to the final product instead of stromal vascular fraction (SVF).[4] PRP, on the other hand, has a much longer history than stromal cells, and many methods are used successfully regarding the effects of growth factors on wound healing and regeneration.[5] In this study, as an innovative alternative to the saline solution used in the indication-based protocols, the process of cutting with sharp blades (adinizing) was performed by combining platelet-poor plasma (PPP) and condensed fat. Thus, by using plasma stromal as a “binder” for cells, the aim was to obtain more cells and greater volume.

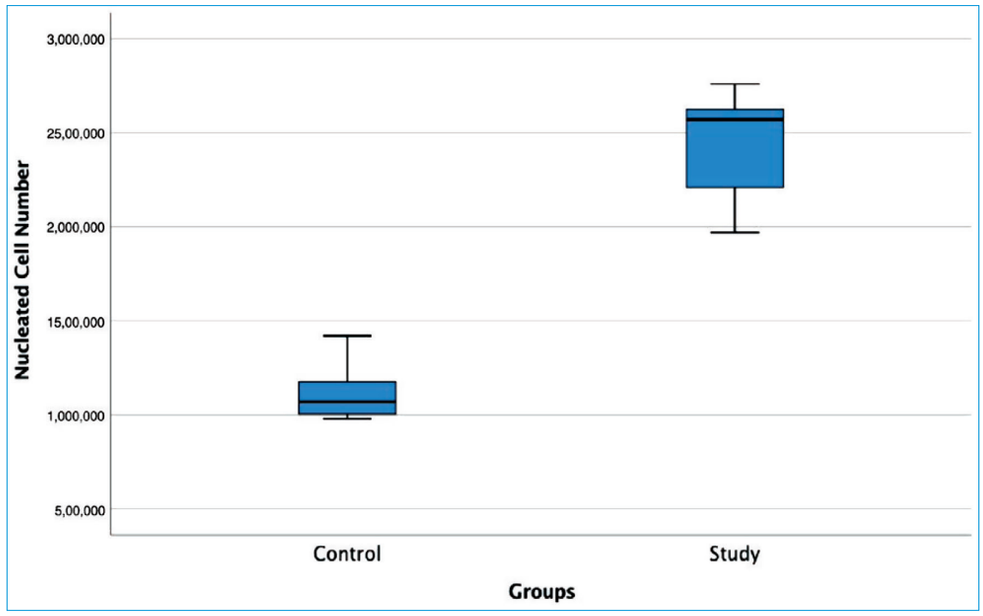
METHODS

This study was conducted according to the standards of good medical practice (ICH-E6) and the principles of the Declaration of Helsinki. All patients were provided detailed information preoperatively, and they gave written consent for all surgical procedures, anesthesia, intraoperative video recording, and photography. In addition, a written consent form was obtained from the patients stating that they willingly donated their adipose tissue for laboratory analysis. This study used a patented CE marking and ISO 13485-certified blade system, and rules of minimal manipulation were followed. No enzymes and similar chemicals were used, and the structure of the fat tissue was not altered. A TriCell PRP kit (Rev-Med Inc, Korea) was used to obtain PPP. Twenty-seven cm3 of venous blood was mixed with 3 cm3 citrates. It was first centrifuged at 3200 rpm for 4 minutes, then at 3300 rpm for 3 minutes, and after the second centrifuge, the PPP in the second chamber of the kit was automatically obtained. Under local anesthesia, 15 cm3 of adipose tissue was harvested from the abdominal area with a 3-mm-diameter 4-hole cannula and then centrifuged at 500 G for 2 minutes, and condensed fat was obtained by discarding tumescent fluid and blood elements.

An estimated 5 cm3 condensed fat was mixed with 5 cm3 PPP in the study group, and 5 cm3 saline in the control group, and then the adinizing process was performed with 2400-µm, 1200-µm, and 600- µm diameter ultra-sharp blades, respectively (Adinizer, BSL-rest, Korea) with 25 back-and-forth movements between the two injectors. Finally, stromal cells were obtained by centrifugation at 1200 G for 5 minutes. The final product, total stromal cells (TOST), was received mainly in liquid form. (See Video [online], MEST preparation.) Total viable nucleated cell recovery and the viability percentage were determined using a LunaStem Automated Fluorescence Cell Counter device (Logos Biosystems, South Korea) with acridine orange/propidium iodide stain in each delivery method before and after the process. After the process was completed, PRP was added to TOST.

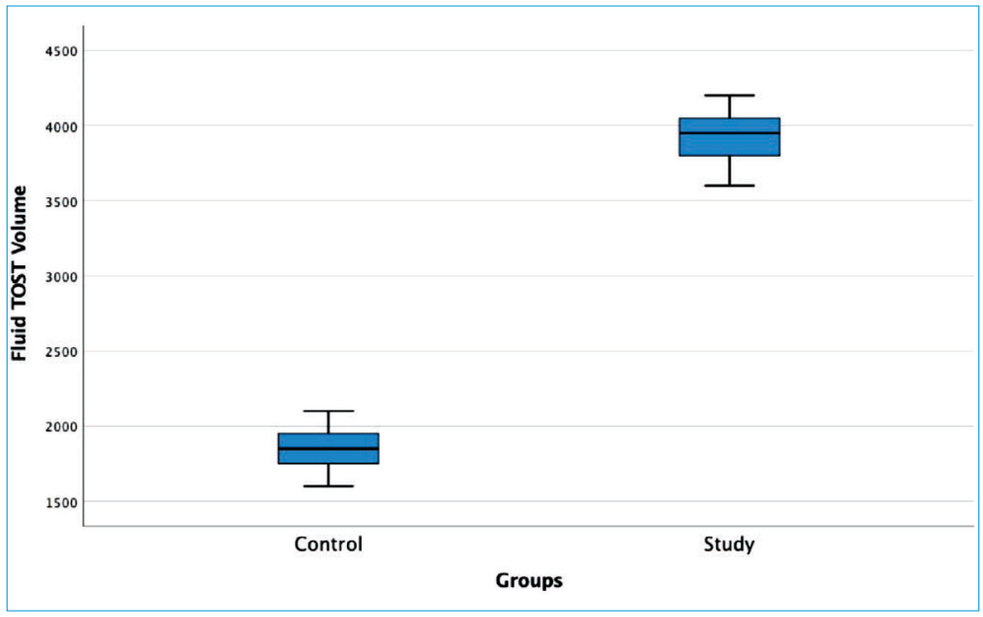
Thus, stromal cells were obtained mechanically from adipose tissue using PPP simultaneously, and a much stronger effect was expected by adding PRP obtained from blood to TOST.

Figure 1. ***Comparison of nucleated cells in milliliters.***



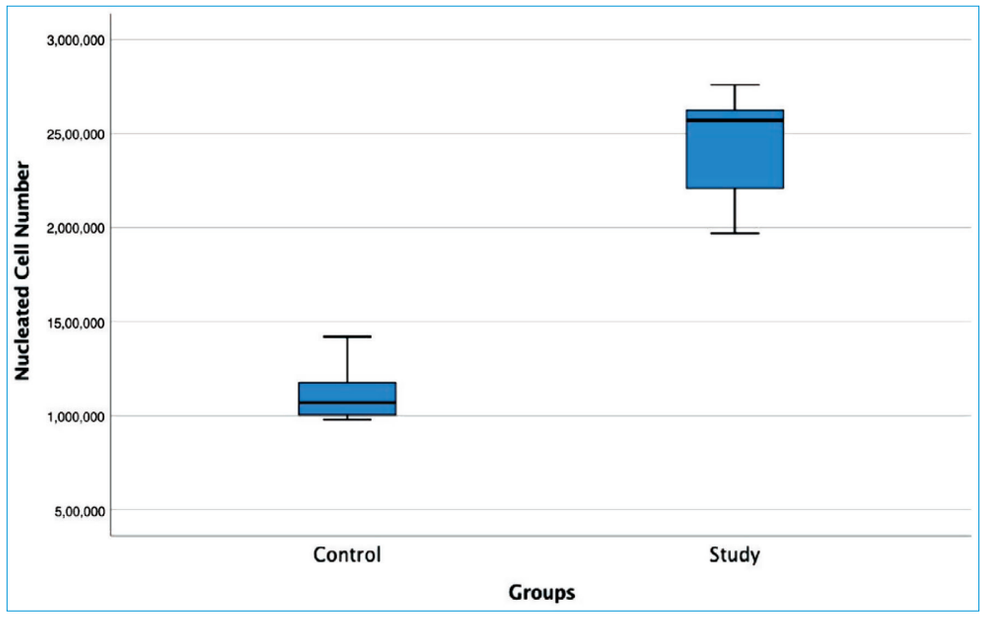
While an average of 1.11 x 106 ± 1.46 × 105 nucleated cells were obtained in the control group, this number was 2.44 x 106 ± 2.99 × 105 in the study group. The 2.2-fold difference between them was statistically significant (<0.001).

Figure 2. ***Comparison of volumes of total stromal cells (TOST).***



While an average of 1.85 ± 0.16 mL TOST was obtained after the procedure in the control group, this volume was 3.92 ± 0.19 mL in the study group. The 2.1-fold difference between them was statistically significant (<0.001).

Figure 3. ***Comparison of total nucleated cells in 10 mL condensed fat.***



When 10 cm3 of condensed fat tissue was taken as reference in the control group, an average of 4.11 × 106 ± 6.78 × 105 stromal cells were obtained after all procedures, while this number was 19.16 x 106 ± 2.58 × 105 in the study group. The 4.7-fold difference between them was statistically significant (<0.001).

RESULTS

Supercharged mechanical stromal cell transfer (MEST) was tested in 8 cases, and results are presented in Figures 1-4. Figure 5 presents components of whole blood and agonized fat after centrifugation.

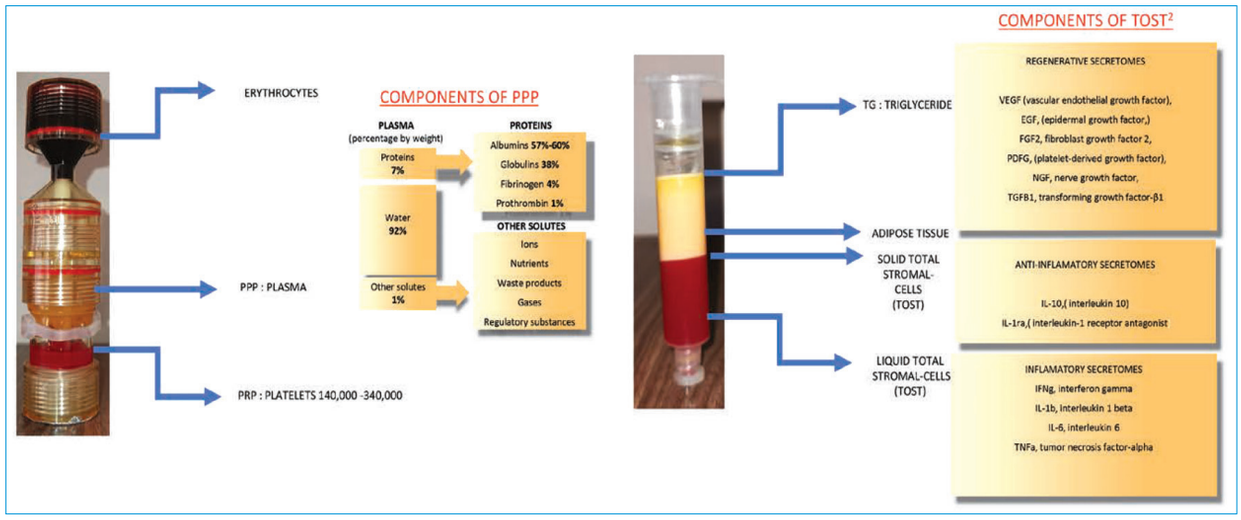
Figure 4. ***Comparison of results of control and study group.***

	Control group	Study group	P
Nucliated Cell Number in ml.	1,11 x 10^6 ± 1,46 x 10^5	2,44 x 10^6 ± 2,99 x 10^5	<0.001
Fluid TOST Volume ml.	1,85 ± 0,16	3,92 ± 0,19	<0.001
Total Nucliated Cell Number in 10 cc Adipose Tissue	4,11 x 10^6 ± 6,78 x 10^5	19,16 x 10^6 ± 2,58 x 10^5	<0.001
Viability (%)	92,25 ± 3,19	92,13 ± 1,56	0.922
Average Nucleated Cell Size (µm)	9 ± 2	8 ± 3	0.896

(The data analysis was done using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY). The descriptive statistics were given as mean ± SD. The normal distribution of the numerical variables was determined using the Shapiro-Wilk normality test. If the data complied

with a normal distribution, the statistical differences between the groups were evaluated using the 1-way analysis of variance and post hoc tests. Mann-Whitney U tests were used if the data did not comply with a normal distribution. A P value of <0.05 was considered to be statistically significant.) The study group found 2.2 times more nucleated cells in 1 mL (<0.001). As a result of the process, TOST was obtained at 2.1 times higher volume (<0.001). When 10 cm3 of condensed adipose tissue was taken as a reference, a total of 4.7 times more stromal cells was obtained (<0.001). There was no statistically significant difference in viability and average cell size in the study and control groups (0.922, 0.896).

Figure 5. ***Components of whole blood and adinized condensed adipose tissue after centrifugation.***



DISCUSSION

When PRP is obtained in conventional applications, the plasma part (called PPP) is discarded, and the PRP part is applied in a broad spectrum due to the growth factors it contains.[5] The clinical application of PRP by combining it with stromal cells obtained from adipose tissue both enzymatically and mechanically is a concept that has been introduced previously.[1,5-7] Stevens et al. described this approach as plate let-rich stroma and reported that it would yield more successful results in androgenic alopecia and osteoarthritis than PRP alone or SVF alone.[1,6] Similarly, Butt et al. obtained stromal cells from adipose tissue mechanically. They emphasized that its combination with PRP provided results far superior to the sole use of PRP.[7] Our study differs from all stromal cell PRP combinations in the literature.[1,5-7] In our research, for the first time, we obtained stromal cells from adipose tissue by mixing 50% of the condensed adipose tissue with PPP before the procedure, mechanically using sharp blades. In the technique described previously by Copcu,[2] indication-based protocols were defined to obtain a higher number of stromal cells in liquid form (conventionally, they are in solid or emulsified fatty consistency) by mechanical stromal cell recovery processes. In this approach, when the adipose tissue was mixed with saline at a rate of 50% before adinizing, more cells and total stromal cells were obtained in liquid form. This may be due to polarity and density. Adipocytes have no positive and negative charged points – the charge

distribution is equal, indicating that they are nonpolar. Nonpolar molecules do not dissolve well in polar structures like water; they tend to repel each other and remain separated, even when shaken vigorously.[8]

However, mesenchymal stromal cells respond to superficial electric charges, unlike adipocytes.[9] The back-and-forth movements described above release the stromal cells when the adipose tissue passes through the metal blades between the two injectors. However, the kinetic energy generated at this time affects the polarity of the cells. In pre-adinizing dilution, this electrical polarity affects the relationship between saline and stromal cells and helps separate stromal cells more successfully. Zimmerlin also described the intra-tracheal route of stromal cells combined with fibrin as a glue.[10] In the innovative approach we are presenting in this study, plasma is used instead of saline. The content of plasma is 7% protein and 4% fibrinogen. Thanks to these structures in the plasma acting as a binder for stromal cells, it is possible to obtain both twice the volume and 4.7 times more stromal cells.

CONCLUSION

At the same time, adding the obtained PRP to this final product will allow the application of "supercharged" cells in a much stronger sense, as described in many studies in the literature. However, advanced clinical studies are required to prove this hypothesis.

REFERENCES

1. Stevens H.P., Donners S., de Bruijn J. Introducing platelet-rich stroma: platelet-rich plasma (PRP) and stromal vascular fraction (SVF) combined to treat androgenetic alopecia. *Aesthet Surg J.* 2018; 38: 811–822.
2. Copcu H.E., Oztan S. New mechanical fat separation technique: ARAT and MEST. *Aesthetic Surg J Open Forum.* ojaa035.
3. Raposio E., Bertozzi N. Isolation of ready-to-use adipose-derived stem cell (ASC) pellet for clinical applications and a comparative overview of alternate methods for ASC isolation. *Curr Protoc Stem Cell Biol.* 2017; 41: 1F.17.1–1F.17.12.
4. Copcu H.E., Oztan S. Not stromal vascular fraction (SVF) or nanofat, but total stromal-cells (TOST): A new definition. Systemic review of mechanical stromal cell extraction techniques. *Tissue Eng Regen Med*. 2021; 18: 25–36.
5. Alves R., Grimalt R. A review of platelet-rich plasma: History, biology, mechanism of action, and classification. *Skin Appendage Disord.* 2018; 4: 18–24.
6. Stevens H.P., van Boxtel J., van Dijck R., et al. Platelet-rich STROMA, the combination of PRP and tSVF, and its potential effect on knee osteoarthritis. *Appl Sci.* 2020; 10: 4691.
7. Butt G., Hussain I., Ahmad F.J., et al. Stromal vascular fraction enriched platelet-rich plasma therapy reverses the effects of androgenetic alopecia. *J Cosmet Dermatol.* 2020; 19: 1078–1085.
8. Boston University School of Public Health. Basic Cell Biology. Available at http: //sphweb.bumc.bu.edu/otlt/MPH Modules / PH/PH709_BasicCellBiology/PH709_BasicCellBIology4.html. Accessed April 20, 2021.

9. Khlusov I.A., Dekhtyar Y., Sharkeev Y.P., et al. Nanoscale electrical potential and roughness of a calcium phosphate surface promote the osteogenic phenotype of stromal cells. *Materials* (Basel). 2018; 11: 978.
10. Zimmerlin L., Rubin J.P., Pfeifer M.E., et al. Human adipose stromal vascular cell delivery in a fibrin spray. *Cytotherapy*. 2013; 15: 102–108.

METABOLIC ENDOTOXEMIA AND MALE INFERTILITY

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SUMMARY

Infertility affects 10-15% of couples of reproductive age. Metabolic syndrome (MetS), especially in younger populations, is a risk factor for fertility disorders. Obesity and high-fat diets disrupt gut mucosal barriers, leading to endotoxemia and systemic inflammation, which negatively impact male fertility. This review examines the relationship between MetS, gut microbiota dysbiosis, and male infertility. Studies show that high-fat diets increase gut permeability and endotoxin levels, impairing spermatogenesis and sperm motility. Probiotics and prebiotics have shown promise in improving gut barrier function, reducing endotoxemia, and enhancing sperm quality. Weight loss and dietary modifications also improve semen quality and reproductive hormones. There is a need for evidence-based preconception nutritional guidance for men. Further research is necessary to explore how diet impacts male reproductive function and to develop effective treatments for idiopathic male infertility using microbiota-targeted therapies.

Keywords: Infertility, metabolic syndrome, gut microbiota, male fertility, endotoxemia, probiotics, dietary modifications.

INTRODUCTION

Infertility is a global health problem affecting 10–15% of couples of reproductive age. Lifestyle factors can impact male fertility through alterations in endocrine profiles, spermatogenesis, and sperm function. Identifying contributing factors to infertility may offer more straightforward and/or more effective therapeutic options than current treatments. The increasing worldwide prevalence of metabolic syndrome (MetS), especially in younger populations, is a risk factor for fertility disorders. Obesity and a high-fat or high-calorie diet can cause a breakdown in the gut mucosal barrier, leading to the passage of gut bacteria membrane remnants into the systemic circulation

and initiating chronic systemic inflammation. This inflammation, particularly in adipose tissue, is implicated in diet and obesity-related insulin resistance.[1] However, a direct correlation between MetS and male infertility remains unclear.

METHOD

Gómez-Elías et al.[2] induced a metabolic syndrome-like condition in (C57BL/6xBALB/c) F1 male mice by feeding them a high-fat diet (HFD, 30% fat) for 19 weeks, while controls received a normal-fat diet (NFD, 6% fat). Ning Ding et al.[3] investigated if HFD-induced gut microbiota dysbiosis could influence spermatogenesis and sperm motility. Fecal microbes from HFD-fed or regular diet (ND)--fed male mice were transplanted to mice maintained on ND. Sperm count and motility were analysed. The study explored how diet impacts male reproductive function and developed evidence-based preconception nutritional guidance for men. Dardmeh et al. investigated the effect of probiotics (Lactobacillus rhamnosus) on sperm kinematic parameters, testicular weight, lipid profiles, and reproductive hormones in male mice. Maretti and Cavallini conducted a placebo-controlled study on the impact of prebiotic/probiotic therapy on testosterone levels and sperm quality in infertile men.

RESULTS

HFD-fed mice exhibited increased body weight, hypercholesterolemia, hyperglycemia, and glucose intolerance, with more gonadal fat, lower epididymal weight, and decreased epididymal sperm count. Sperm analysis showed significant differences between HFD- and NFD-fed mice in sperm count, viability, morphology, and motility. Transplantation of HFD gut microbes into ND-maintained mice significantly decreased spermatogenesis and sperm motility and increased proinflammatory cytokines in the epididymis.[3] Obesity and high-fat diets result in changes to gut bacteria and increased intestinal permeability, leading to metabolic endotoxemia. Kelton Tremellen postulated that bacterial lipopolysaccharide (LPS) from the gut lumen into circulation is a critical inflammatory trigger underlying male hypogonadism.[4] Linn B. Hakonsen et al. observed that weight loss improved semen quality.[5] Karma L. Pearce et al. found that metabolic endotoxemia and its associated oxidative stress may drive sperm DNA damage in obese men.[6] Dardmeh et al.[7] showed probiotics could eliminate obesity's adverse effects on semen quality. Everard et al.[8] found prebiotic treatment improved gut barrier function and metabolic parameters. Valcarce et al.[9] demonstrated probiotics improved sperm quality in asthenozoospermic men. Maretti and Cavallini[10] reported a significant improvement in testosterone levels and sperm quality with prebiotic/probiotic therapy.

DISCUSSION

Obesity and a high-fat/high-calorie diet cause changes in gut bacteria and intestinal permeability, leading to metabolic endotoxemia and systemic inflammation, negatively affecting male fertility. High-fat diets are more efficient in transporting bacterial endotoxin from the gut lumen into circulation. The relationship between diet composition and obesity involves interactions between dietary macronutrients. There is a clear need to explore further how diet impacts male reproductive function to develop evidence-based preconception nutritional guidance. Probiotics and pre-

biotics can improve gut barrier function, reduce metabolic endotoxemia, and positively impact sperm quality and reproductive hormones.

CONCLUSION

Obesity and high-fat/high-calorie diets contribute to metabolic endotoxemia and systemic inflammation, negatively affecting male fertility. Lifestyle changes, including weight loss and dietary modifications, can improve semen quality and reproductive hormones. Probiotics and prebiotics offer potential therapeutic options for improving male fertility by enhancing gut barrier function and reducing metabolic endotoxemia. Further research is needed to develop evidence-based nutritional guidance for men to improve reproductive outcomes.

REFERENCES

1. Mehta NN, McGillicuddy FC, Anderson PD, Hinkle CC, Shah R, Pruscino L, Grivennikov SI, Ley K, Mosser DM, Miller LS, Toliver-Kinsky T, de Souza LS, Ferreira RA, Sierra JC, Balter A, Mandell L, Schlaepfer DD, Tabas I, Stoffel M, Reilly MP. Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. *Diabetes*. 2010; 59(1): 172-81. doi:10.2337/db09-0367
2. Gómez-Elías MD, Rodríguez-González GL, Ávila-González CC, Lara-Padilla E, Rivas-Arancibia S, Granados-Badillo A, López-Meza JE, Jiménez-Estrada M, Ortiz-Lazareno PC, Pérez-Camarillo S. Association between high-fat diet feeding and male fertility in high reproductive performance mice. *Sci Rep.* 2019; 9: 18546. doi:10.1038/s41598-019-54799-3
3. Ding N, Zhang X, Zhang XD, Zhang C, Jin J, Ma YH, Dong JY, Zhang YL, Wang YX, Ma ML, Guo YX, Huang CJ, Wei Y, Wang LH, Jin DM, Yang QL. Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. *Gut*. 2020; 69(9): 1608-1619. doi:10.1136/gutjnl-2019-319127
4. Tremellen K, Pearce K. Dysbiosis of Gut Microbiota (DOGMA) – a novel theory for developing Polycystic Ovarian Syndrome. *Basic Clin Androl.* 2016; 26: 7. doi:10.1186/s12610-016-0032-1
5. Hakonsen LB, Thulstrup AM, Aggerholm AS, Olsen J, Bonde JP, Andersen CY, Bungum M, Ernst EH, Hansen ML, Gernow A, Ramlau-Hansen CH. Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reprod Health.* 2011; 8: 24. doi:10.1186/1742-4755-8-24
6. Pearce KL, Tremellen K, Lopez-Villalobos N, Kang HJ, MacDonald C, Sheedy JR. Obesity-related metabolic endotoxemia is associated with oxidative stress and impaired sperm DNA integrity. *Basic Clin Androl.* 2019; 29: 6. doi:10.1186/s12610-019-0087-y
7. Dardmeh F, Alipour H, Gazerani P, Vaegter HB, Alipour B, Nielsen HI. Lactobacillus rhamnosus PB01 (DSM 14870) supplementation affects markers of sperm kinematic parameters in a diet-induced obesity mice model. *PLoS One.* 2017; 12(10) doi:10.1371/journal.pone.0185964
8. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD. Cross-talk between Akkermansia muciniphila

and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A*. 2013; 110(22): 9066-9071. doi:10.1073/pnas.1219451110

9. Valcarce DG, Herráez MP, Robles V. Probiotic administration improves sperm quality in asthenozoospermic human donors. *Benef Microbes.* 2017; 8(2): 193-206. doi:10.3920/BM2016.0142
10. Maretti C, Cavallini G. The association of a probiotic with a prebiotic (Flortec, Bracco) to improve the quality/quantity of spermatozoa in infertile patients with idiopathic oligoasthenoteratospermia: a pilot study. *Andrology*. 2017; 5(3): 439-444. doi:10.1111/andr.12340

ARTIFICIAL ABORTION – THE GEORGIAN STORY

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SUMMARY

This chapter provides a comprehensive overview of the current state of abortion and contraception in Georgia, highlighting the unique characteristics that set them apart from other regions. The second section explores the principles of eliminating restrictive policies on abortion and contraception. The final and main topic presents the author's profound thoughts and philosophy on abortion and life issues, inviting the reader to engage in thoughtful discussion.

Keywords: Artificial abortion, Contraception, Reproductive health

INTRODUCTION

The reader may wonder: Why the Georgian Story?

1. Maybe because Georgia is the first and the only country in the world where Reproductology, the science about both women's and men's reproductive health, has widely been recognized as the officially independent medical discipline, starting since 1997, not just the part of obstetrics and gynecology.
2. Georgia possesses a nonofficial record in the fast spreading of modern contraception. In any case, according to the research of the Zhordania Institute of Reproductology (the oldest clinic of this type in the world, established in 1958) in Georgia, in 1987, when the population averaged 5.5 million, the artificial abortion total rate was 300,000, two-thirds of which was illegal, approximately 2-4 abortions per woman. In this period, Georgia was a part of the Soviet Union, which was the first in the world abortion statistics, and Georgia was one of the leaders among the Soviet republics. Furthermore, the usage of modern contraception was 0 (zero), which means that contraception, as the regulatory method

of reproductive function, did not exist in our country. By the year 2010, the prevalence of modern contraception exceeded 70%. We consider this jump from 0 to 70 per cent a unique fact, and we rely on the Zhordania Institute's studies but not the CDC's data, which we consider unreliable since the survey was carried out with serious omissions: men were not included in the research.

3. We take great pride in our country being the one in the Christian world where complete understanding has been achieved between the Church and reproductive specialists, a successful collaboration that can serve as a model for other regions. For instance, no significant issues were encountered during In Vitro Fertilization (IVF) development and implementation. All those mentioned above result from permanent and frequent consultations between the Georgian Orthodox Church and the Zhordania Institute of Reproductology. Our experience will benefit Christian countries struggling to develop and use the main principles of reproductive health.

Regarding the contemporary dynamics of abortion and contraception, our experience points out that the abortion rate is 10,000, and the use of contraception reaches up to 90%. It may be said that the unpleasant phenomenon of abortion is defeated in Georgia. The same is shown in the statistical analysis of recent years (Unfortunately, only until 2010. After this, no research has been conducted due to the government's unstable political situation and inattention) and in the results of interested specialists' permanent surveillance in the Zhordania Institute. To be more exact, artificial abortion has not been eliminated. Disappointingly, the abortion rate is 10 to 12 thousand per year[3], which is relatively high for a country with a population of 3.7 million. It must be noticed that illegal abortions are eliminated. The rest of the registered abortion rate has steadily positive dynamics, which means it is reducing. Here, it must be noted that the main reason for this achievement in our country is the comprehensive implementation of contemporary contraceptives by the Zhordania Institute, a reassuring sign of progress. It may not be ignored that local media representatives were helpful in this process and are still helping us. We appreciate the Ministry of Health, Labor, and Social Affairs of Georgia's noninterference in our oversight areas.

The position of our Church is critical as well, which believes that abortion is a big sin and must be eliminated. This opinion is wholly shared, with the added belief that abortion is detrimental to a woman's health and should be abolished. As for contraception, the Georgian Christian Orthodox Church also considers it a sin but "less sin than abortion." This kind of assessment is, at this time, acceptable for us, the reproductologists, especially, because it does not make an accent on abortion and its administrative prohibition or reduce the usage of contraception, which, of course, is the result of our explanations, based on the facts of the world experience.

METHODS

Data were sourced from the Zhordania Institute of Reproductology, the oldest clinic of its type in the world, and other relevant Georgian health statistics. The analysis covers periods when reliable data were available, particularly up to 2010, as subsequent data collection was hindered by political instability.

The study focuses on the historical and current prevalence of artificial abortions and contraception use in Georgia. It includes qualitative assessments based on consultations between reproductive specialists and the Georgian Orthodox Church.

Quantitative data were gathered from national statistics and Zhordania Institute records. Qualitative data were collected through interviews and consultations with key stakeholders, including healthcare providers and church representatives.

RESULTS

Historical Context and Current Statistics

In 1987, Georgia, then part of the Soviet Union, had an artificial abortion rate of 300,000 annually, with two-thirds being illegal. Modern contraception was non-existent at that time. By 2010, the prevalence of contemporary contraception had increased to over 70%, leading to a significant reduction in abortion rates. As of the latest reliable data, the abortion rate is approximately 10,000 to 12,000 per year, with illegal abortions primarily eliminated.

Role of Contraception and Church-State Collaboration

The successful reduction in abortion rates is attributed to the widespread adoption of modern contraceptives and effective collaboration between reproductive health specialists and the Georgian Orthodox Church. The Church, while considering both abortion and contraception as sins, has worked with health specialists to prioritize contraception as a lesser evil compared to abortion.

Impact of Policies on Abortion Rates

Evidence from Georgia and other post-Soviet countries indicates that restrictive abortion laws do not reduce the number of abortions but increase the incidence of unsafe, illegal procedures. The introduction of contemporary contraceptives and public education has been crucial in reducing abortion rates in Georgia.

DISCUSSION

Abortion as a Public Health Issue

Today, there is no doubt in competent specialists that in the sphere mentioned above, any prohibition does not bring any result and does not change the abortion rate but increases the number of illegal and nonmedical artificial abortions only. The latter leads to an increase in maternal mortality and morbidity rates, as evidenced by the bitter experiences of post-Soviet countries, Romania, Ireland, Poland, and others. Evidence shows that restricting access to abortions does not reduce their number.1 Countries with highly restrictive abortion laws have a significantly higher proportion of unsafe abortions compared to those with more liberal laws.2 Besides, paradoxical is the fact that the administrative prohibition of abortion causes the rise of so-called Gynecological Tourism. The women, for the need and reason of abortion, travel to other countries where the procedure is permitted. In addition, the world study showed that in countries where abortion was restricted, the proportion of unintended pregnancies ending in abortion had increased. However, it decreased in countries where abortion is broadly legal.[1] It is believed that abortion should not

be prohibited but rather eliminated through the introduction of modern contraceptives, comprehensive public information, and adequate education. Until abortion remains the reality of our lives, talking about its prohibition is detrimental. It is necessary to speak about the harm it brings to a woman's health. At the same time, a temporary introduction of modern alternatives to artificial abortion is required. In Georgia, such a temporary alternative has become the so-called Mini-Abortion (Vacuum Aspiration Procedure), which is much more harmless for the woman's health compared to traditional surgical abortion. It had served its purpose, but by 2000, it was decided that Mini-Abortion had become outdated. Consequently, Medical Abortion was introduced, as it is less harmful to women, more cost-effective, and does not require hospitalization.

Our consideration of any abortion is negative, and it must be eliminated, not by prohibition and forcefulness, but only through explanation, interpretation, promotion of relevant knowledge, and education. We are sure that if there is anyone who hates abortion, that is us, the doctors of the field of Reproductology, in the first place. The reasonable position of the Georgian Association of Reproductive Health is that in recent years, the attitude of our country towards abortion and family planning has been fair and proper and should be continued as long as the results are evident.

Ethical and Philosophical Considerations

We are often asked: is abortion a murder? The response is delivered with firm determination: Yes, abortion is murder because human life begins from its conception. This kind of answer is conducive to the second, natural question: Should the killing doctors who carry out abortions be punished? Our definite answer is: "No" if the doctor does the procedure altruistically, only when he is sure the patient has the vital, medical, and social contraindications for pregnancy. Additionally, the doctor must thoroughly explain everything to the patient to persuade her to maintain the pregnancy.

All this does not relieve the doctor from murder responsibility!

Due to the unfair situation, and after extensive consideration, a philosophy of antenatal life was developed with the hope that, along with the elimination of abortion, the practical necessity for this philosophy will eventually disappear. We are probably not comforting ourselves or imposing our opinions on anybody.

Nevertheless, let us introduce our thoughts on the readers' theoretical and practical assessment.

As already mentioned, we accept the suggestion that all types of artificial abortion are thought to be the facts of life termination and murder. However, it must also be noted that, in our opinion, life is of two types: antenatal and postnatal. Postnatal life begins after delivery, and it belongs to the newborn. Artificial interruption of this life is the greatest crime and is judged accordingly. Antenatal life differs qualitatively from postnatal life because it belongs to the fetus and the mother. As the fetus is not capable of deciding for God, the responsibility for the sin must be placed on the mother and not on the doctor, who is forced into having an abortion. Is it necessary to judge the mother for the crime, or is it not a separate issue? We think that artificial abortion is the mother's sin but not a crime, and only God can judge its level.

Presumably, everybody agrees that there are many such facts in our lives, the fair definition of which exceeds our thinking ability. The rank-and-file cannot analyze such facts but put their trust in God's will. One of them is the still-existing abortion. We have repeatedly clarified that the abovementioned theory is the product of our subjective thinking, which we are not imposing on anybody.

Readers and colleagues are encouraged to consider the fairness and objectivity of this theory.

CONCLUSION

1. Abortion should be eliminated but not prohibited or restricted.
2. Informing the population of modern contraception and its education in this connection must be made essential.
3. Abortion is indeed murder, but in this particular situation, it is a sin rather than a crime.
4. Considering our theory about dividing life into "Antenatal" and "Postnatal" types, all the sins of abortion should be placed on the mother, but not on the doctor.

Declarations of interests: We declare no competing interests.

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We alone are responsible for the views expressed in this article, and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

REFERENCES

1. Bearak J, Popinchalk A, Ganatra B, et al. Unintended Pregnancy and Abortion by Income, Region, and the Legal Status of Abortion: Estimates from a Comprehensive Model for 1990-2019. Lancet Glob Health. 2020;8(9). doi:10.1016/S2214-109X(20)30315-6.
2. Ganatra B, Gerdts C, Rossier C, et al. Erratum: Global, Regional, and Subregional Classification of Abortions by Safety, 2010–14: Estimates from a Bayesian Hierarchical Model. Lancet. 2017; 390(10110):2372-2381. doi:10.1016/S0140-6736(17)31794-4.
3. National Statistics Office of Georgia. Healthcare. https://www.geostat.ge/en/modules/categories/54/healthcare.

PREGNANCY AND RISKS IN PATIENTS 35+

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SUMMARY

The article presents the results of a retrospective and prospective study: analysis of fertility, Caesarean section frequency, assessment of the course of pregnancy and childbirth, the state of the fetoplacental system in primi- and multiparous women of various age groups – 18-25, 26-28, 29-30, 31-35, 36-40 years, older than 40 years. It is shown that the increase in the number of births is due to the rise in fertility only among patients older than 30 years. At the same time, there is a significant decrease in fertility – by 1.3 (due to the age subgroup of 18-25) among younger women. At the same time, an increase in the number of births in older patients occurred in all three groups of observations (31-35, 36-40, over 40 y.o.) by 1.7-2.1. The increase in the CS frequency (from 1998 to 2008) in the age group under 30 years is due to the rise in this indicator only in women aged 26-28 and 29-30. In the group of patients over 30, the increase in the CS frequency concerned only the age groups of 31-35 and 36-40 years(by 1.6 and 1.3). With the age of the patients, the frequency of gestosis, the severity of fetal growth restriction and the frequency of premature birth increased. The duration of labor in multiparous patients in all age groups identified by us did not depend on the interbirth interval and, therefore, the large time interval between labor itself should not serve as a basis for expanding the indications for planned Caesarean delivery.
Keywords: Birth rate, caesarean section frequency, pregnant and parturient women younger and older than 30

INTRODUCTION

The course of pregnancy and childbirth depends on many factors, among which a patient's age is not less important. Many authors consider the age of a woman in labor under 18 and over 30-35 years old as a risk factor for a high incidence of complications for both mother and child.[3, 4, 5, 12] At the same

time, several researchers do not find a significant difference in the frequency of pregnancy complications in women of different age groups.[9, 11]

Most of the studies on the influence of the age factor on the course and outcomes of childbirth concern primiparous women of the older age group. However, there is still no standard view regarding the age "barrier" that would indicate an increased risk of pregnancy complications and childbirth and determine the development of optimal management tactics. The approach to the allocation of groups of patients of "elderly" age is ambiguous: women who give birth for the first time at the age of 30 and older are considered to be such, according to the foreign literature – older than 35 years.[1, 6, 7] There is no finding on the influence of age on the course of pregnancy, childbirth, and perinatal outcomes in multiparous patients, and there is no information about the value of the interbirth interval in them. There is also no precise data on the birth rate in various age groups.

In connection with the above, we conducted a fertility analysis, a comprehensive assessment of the course of pregnancy and childbirth, and the state of the fetoplacental system in first-time and repeat-giving patients of different age groups in two maternity institutions in Moscow.

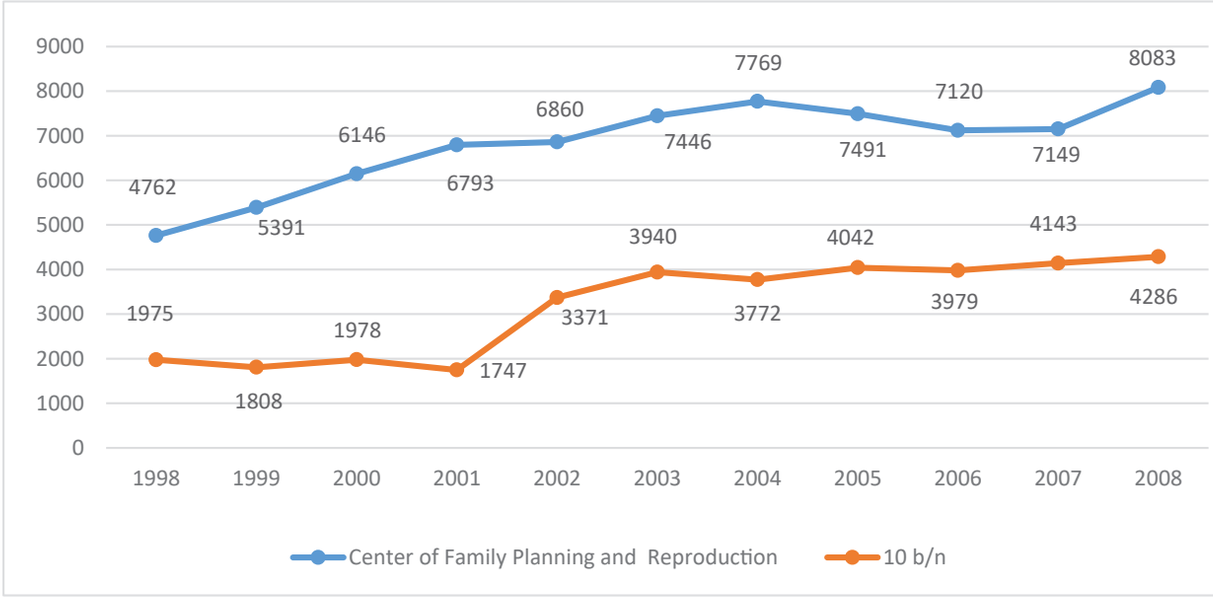
MATERIAL AND METHODS OF RESEARCH

In the first stage, to analyze fertility and study the peculiarities of pregnancy and childbirth in patients of different age groups, we conducted a retrospective analysis of the birth histories of 79,600 patients(46,067 primiparous, 33,533 multiparous women). In the process of retrospective research, we identified the following age groups: 18-25, 26-28, 29-30, 31-35, 36-40, and above 40 years.

The prospective study group consisted of 709 patients (277 – primiparous, 432 – multiparous women) with a similar division to age groups. The frequency of premature and delayed labor, gestoses of various severity, fetal growth resrtriction, labor anomalies, Caesarean section (planned/emergency) were analyzed. The examination of pregnant women in the prospective group, in addition to general clinical and laboratory methods, included ultrasound fetometry and placentometry, dopplerometric assessment of blood flow in the mother-placenta-fetus system, and cardiotocography. The peculiarities of the course of childbirth and the duration of childbearing in different age groups were evaluated, considering parity and, in addition, in multiparous women – depending on the interbirth interval.

RESEARCH RESULTS AND THEIR DISCUSSION

There has been a clear trend towards an increase in the birth rate in recent years. Thus, in Moscow in 2008, the number of births was 104,876, which was 35,902 more than in 1998. In 2008, the number of births reached 8,083, which is 1.7 times higher than in 1998 in the Center of Family Planning and Reproduction, and in maternity hospital No. 10, the number of births increased by 2.17 times over 10 years (Fig. 1).



Picture 1. ***Dynamics of the number of births in the Center of Family Planning and Reproduction and Maternity Hospital No. 10***

We have identified significant differences in fertility dynamics among patients of different age groups. The increase in the number of births is due to the rise in fertility only among patients older than 30 years (from 24% in 1997 to 43.2% in 2008), while among younger women, fertility significantly decreases by 1.3 times (Fig. 2).

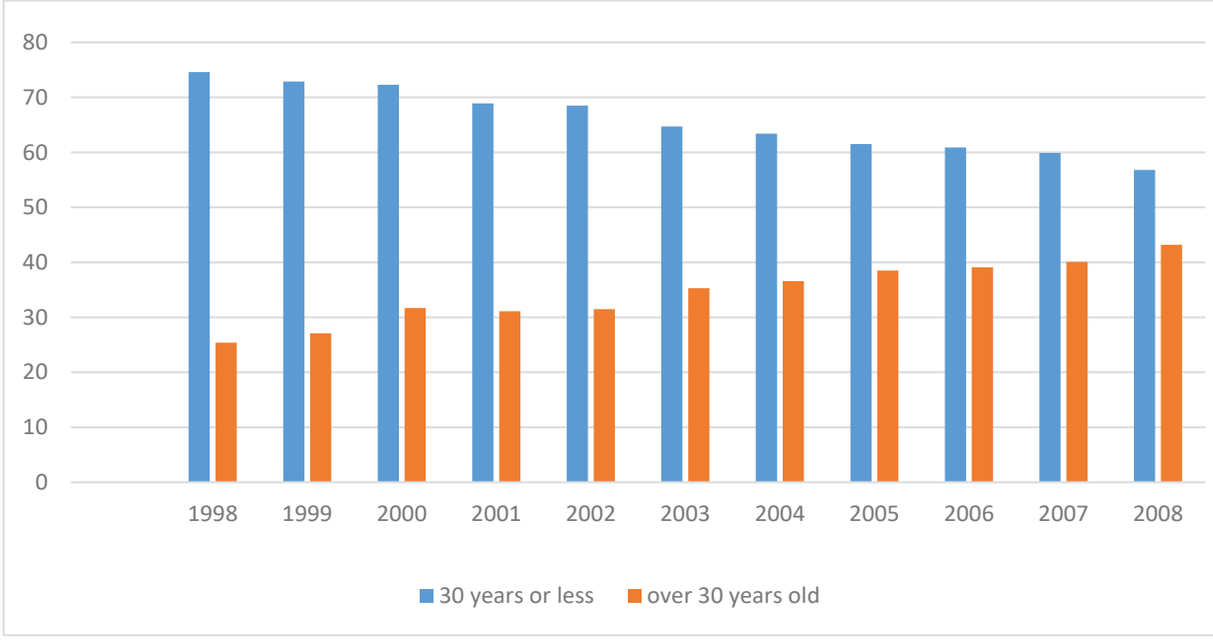


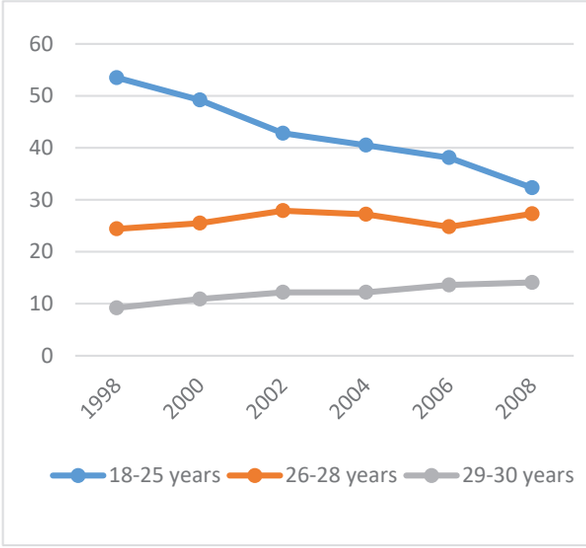
Figure 2. ***Distribution of parturient women depending on age (1998-2008)***

A more detailed analysis of the birthrate dynamics, considering the identified age groups (18-25, 26-28, 29-30, 31-35, 36-40, and above 40 years), showed that it is not quite right to talk about a decrease in the birthrate in the group under 30: a decrease (by 2 times) in the birthrate among patients under 30 years of age occurred only in the age group of 18-25. At the same time, an

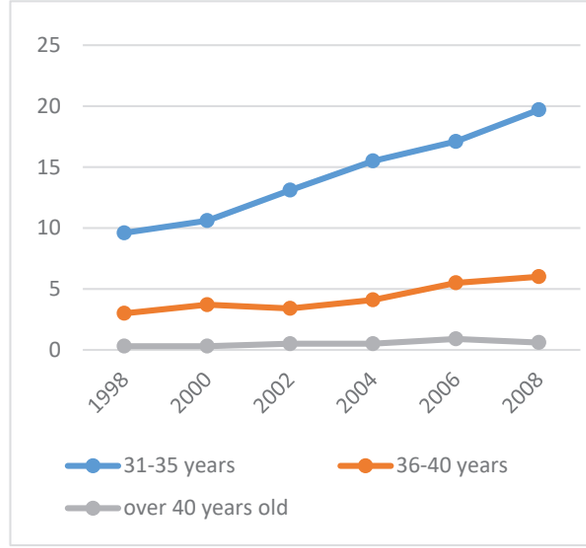
increase in the number of births in older patients happened in all three groups of observations (31-35, 36-40, over 40 years old) by 1.7-2.1 times.

If we analyze the dynamics of fertility according to the parity with the standard division of patients younger and older than 30 years, then the decrease in the number of births among patients younger than 30 years was typical for both primi-and multiparous women from 87.8 to 73.7%% and from 55.3 to 37.1%%, respectively. The increase in the number of births in older patients also did not depend on parity and increased both in primiparous from 12.2 to 26.3%%, and in multiparous women – from 44.7 to 62.9%%.

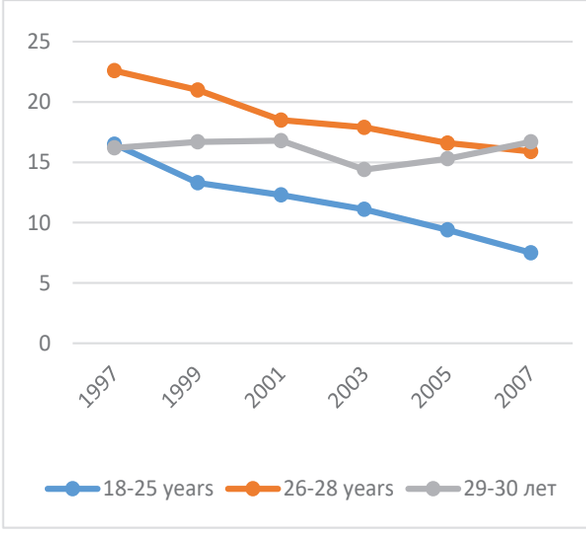
At the same time, a more detailed analysis of the birthrate dynamics in women of the proposed age groups, depending on the parity, revealed important patterns (Fig. 3).



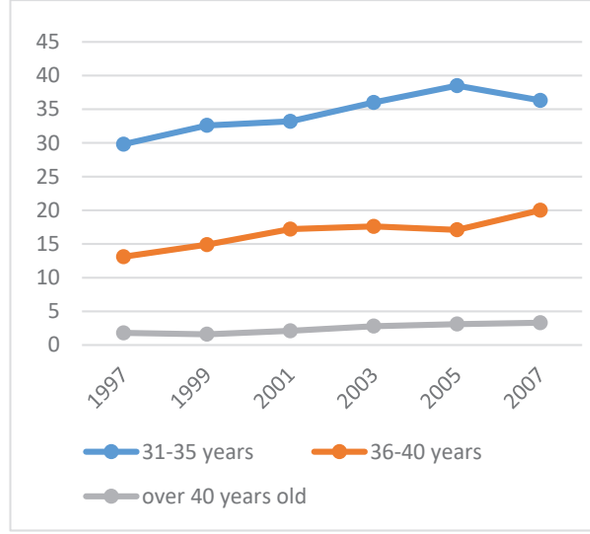
Primiparous women under 30 years old



Primiparous women above 30 years old



Multiparous women under 30 years old



Multiparous women above 30 years old

Figure 3. ***The number of births, the age of parturient women and parity***

As can be seen from Picture 3, if among multiparous women younger than 30 years, a decrease in the number of births by 2008 was observed in all the groups(18-25, 26-28, 29-30 years), then

in primiparous women, a decrease (by 1.7 times) in the number of births was characteristic only for patients 18-25 years old. As for the older age group, the revealed irregularity – an increase in the number of births – did not depend on parity and was observed in all groups (31-35, 36-40, over 40 years old).

As for operative delivery, with a steadily increasing overall frequency of CS, the growth of this indicator from 1998 to 2008 in the group of patients above 30 years and under 30 years was almost the same – by 1.4 and 1.3 times, respectively (Fig. 4).

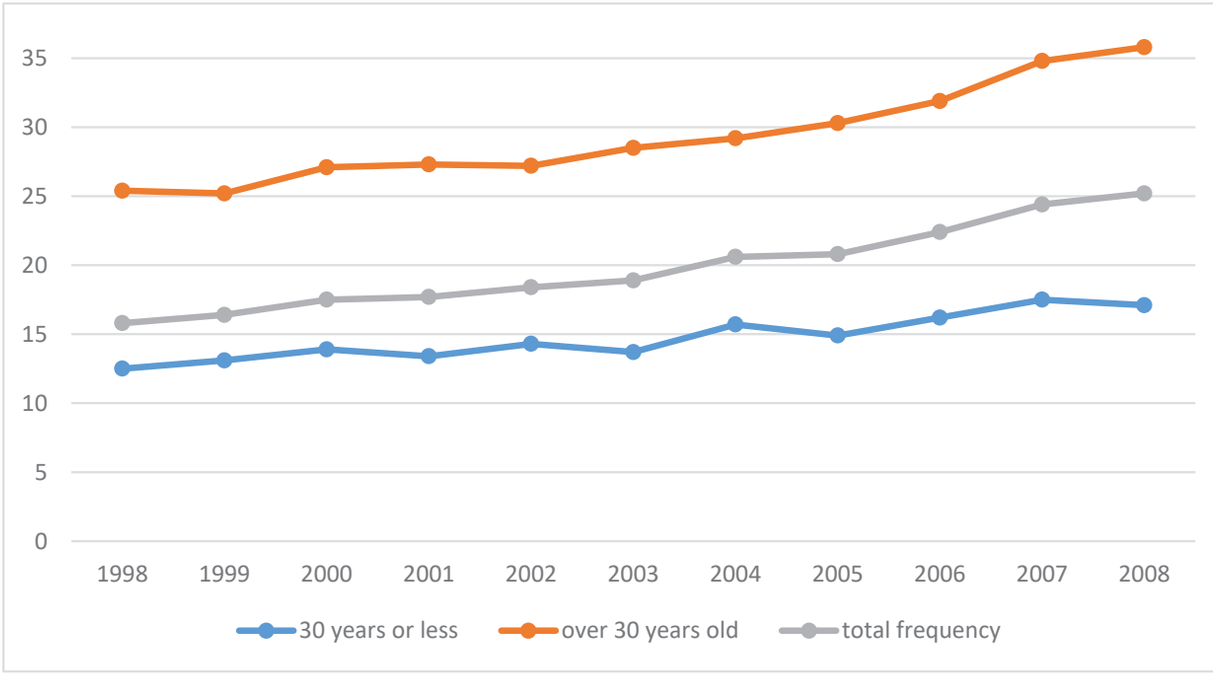


Figure 4. ***Frequency of CS and age of maternity***

At the same time, it is interesting to note that the percentage of primiparous and multiparous women among all those delivered by CS has undergone significant changes over 10 years. Thus, if in 1998 the share of multiparas was only 33.7%, then in 2008, this indicator increased to 48.7%; that is, among the patients operated on in 2008, the number of primiparas and multiparas was almost the same. The increase in the frequency of CS in multiparous women can be explained, first of all, by the rise in the number of patients with a uterine scar.

A more detailed analysis with consideration of the identified age subgroups showed that the increase in the frequency of CS (from 1998 to 2008) in the age group under 30 years is related to the rise in this indicator only in women 26-28 and 29-30 of age (by 1.25-1.3 times). In the group of patients over 30 years of age, the increase in the frequency of CS was only concerned with the age groups of 31-35 and 36-40 years(1.6 and 1.3 times). In patients aged 18-25 and over 40, the frequency of CS practically did not change (10.6 – 11.9% and 45.2-44.6% respectively).

Further, as part of a prospective study (709 patients), we analyzed the course of pregnancy, childbirth, and perinatal outcomes in patients considering the age categories we identified: 18-25, 26-28, 29-30, 31-35, 36-40, and above 40.

The course of pregnancy in the prospective group was complicated by gestosis of varying severity in 6.6% of patients. The analysis of this complication frequency, considering the se-

lected age groups, showed a direct correlation between the frequency of gestosis and the age of patients. Thus, the frequency of gestosis in patients 18-25, 26-28, 29-30, 31-35, 36-40 and older than 40 years was respectively 4,7 %, 5,6 %, 6,3 %, 7,9 %, 8,4 %, 8,5 % and it did not depend on the degree of its severity. Ebclab U. and Vilpa T (1994) also indicate a higher frequency of gestosis (by 2 times) in women above 30-35 years, although the authors cite higher rates of this complication.[8] At the same time, Z. Kozinszky et al. (2002) and B. Sibai et al. (1997) in their studies showed that the age of a woman is not a risk factor for the development of gestosis.[10, 13]

We did not find a clear dependence of the frequency of placental insufficiency and fetal growth retention on the age of primiparous women. At the same time, a more pronounced degree of fetal growth retardation (2-3 degrees) among patients above 35 years attracted attention in multiparous women. At the same time, it should be noted that in the group multiparous of 36-40 years, the ratio of fetuses with growth restriction of 2 and 3 degrees was 63.6% and 36.4%. In patients over 40, these indicators were 71.4% and 28.6%; that is, fetus growth restriction of 3rd degree was observed more. This is, to a certain extent, consistent with the findings of Belousova V.S. (2004), who showed that fetus growth restriction of 2 and 3 grade was more common (1.9 and 2.2 times) in the group of patients above 30 years.[2]

With an increase in the age of the examined patients, the frequency of pre-term labor significantly increased, amounting to 4.3% in the group of 18-25 years and 12.4% in women over 40 years (Fig. 5). This fact can be explained by a higher frequency of multiple pregnancies (5.2%) in the group of patients older than 40 years. And even excluding multiple pregnancies, the frequency of premature births in them exceeded those in patients below 30 years by more than 2 times.

It is important to note that the frequency of premature birth was the highest in primiparous mothers over 40 years old, almost 4 times higher than in multiparous patients of the same age.

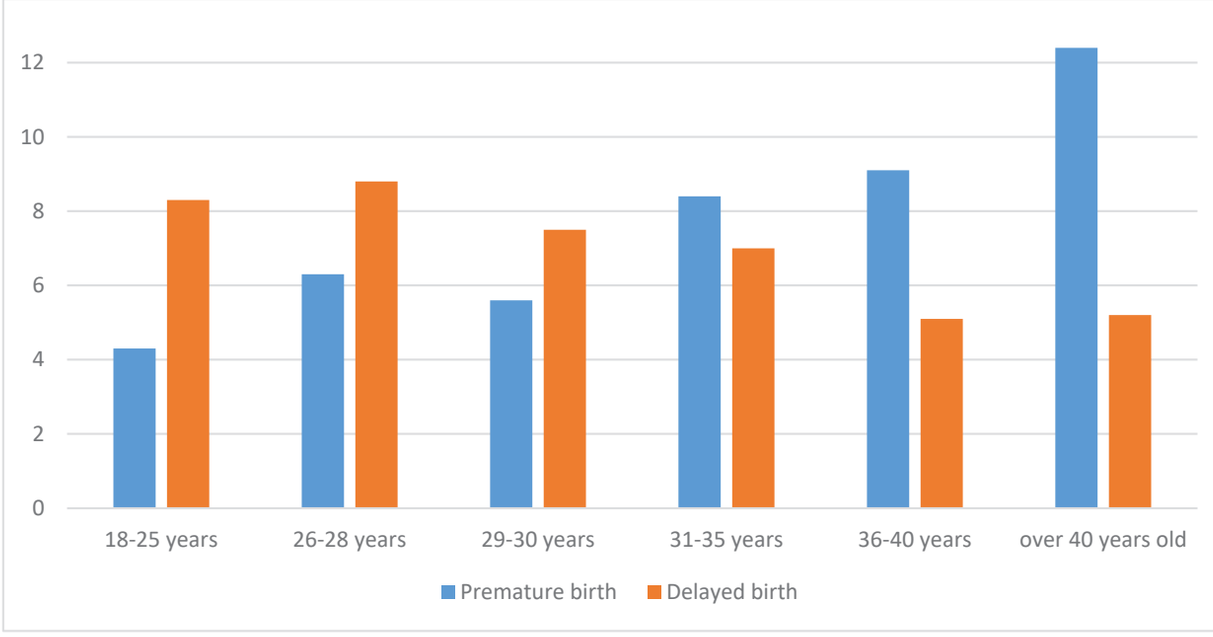


Figure 5. ***The frequency of premature and delayed births in different age groups.***

Unlike preterm labor, the frequency of delayed labor, as seen in Picture 5, was the highest in patients 18-25 and 26-28 years old. The lower frequency of delayed delivery in patients over 28 years of age can be explained by closer attention to this ("older") group of pregnant women, active management tactics: prenatal hospitalization and cervix preparation for childbirth, and an increase in indications for planned Caesarean section, which did not allow delayed birth.

Considering parity, the labour duration in primiparous women ranged from 7h 10 min. to 8 h 50 min., and in multiparous births—from 5 h 5 min. to 6 h 50 min. Of interest is our analysis of labor duration in multiparous patients depending on the intergenetic interval (Fig. 6).

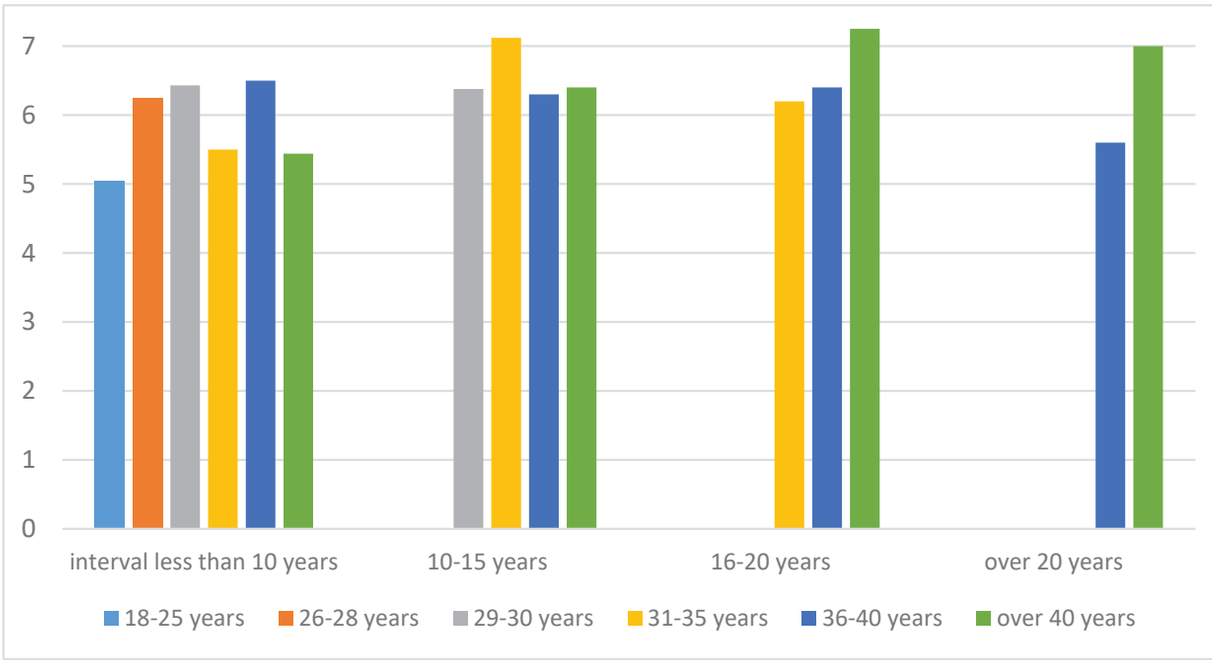


Figure 6. ***Duration of labor and the interbirth interval in patients of different age groups.***

As our studies have shown, the time interval between deliveries did not affect their duration and frequency of labor strength. Even with an interval of more than 20 years (21-24 years), the duration of labor ranged from 5 to 8 hours.

This contradicts the generally accepted opinion that a long interbirth interval is a risk factor for an increase in the duration of labor, the frequency of labor strength, etc., which often serves as one of the main indications for a planned Caesarean section even in the absence of a complicated course of pregnancy and childbirth.

As expected, the frequency of Caesarean sections in the prospective group of patients correlated with age and increased among both primer– and multiparous patients (Fig. 7).

At the same time, it should be noted that in patients 18-30 years old, the frequency of CS practically did not depend on parity, whereas in the age groups of 31-35, 36-40, and over 40, the frequency of Caesarean section was higher in primiparous mothers.

With the age of patients, the frequency of planned Caesarean section increased from 31% in 18-25 years to 44.2% in women over 40 years old, which is explained by the expansion of indications for abdominal delivery.

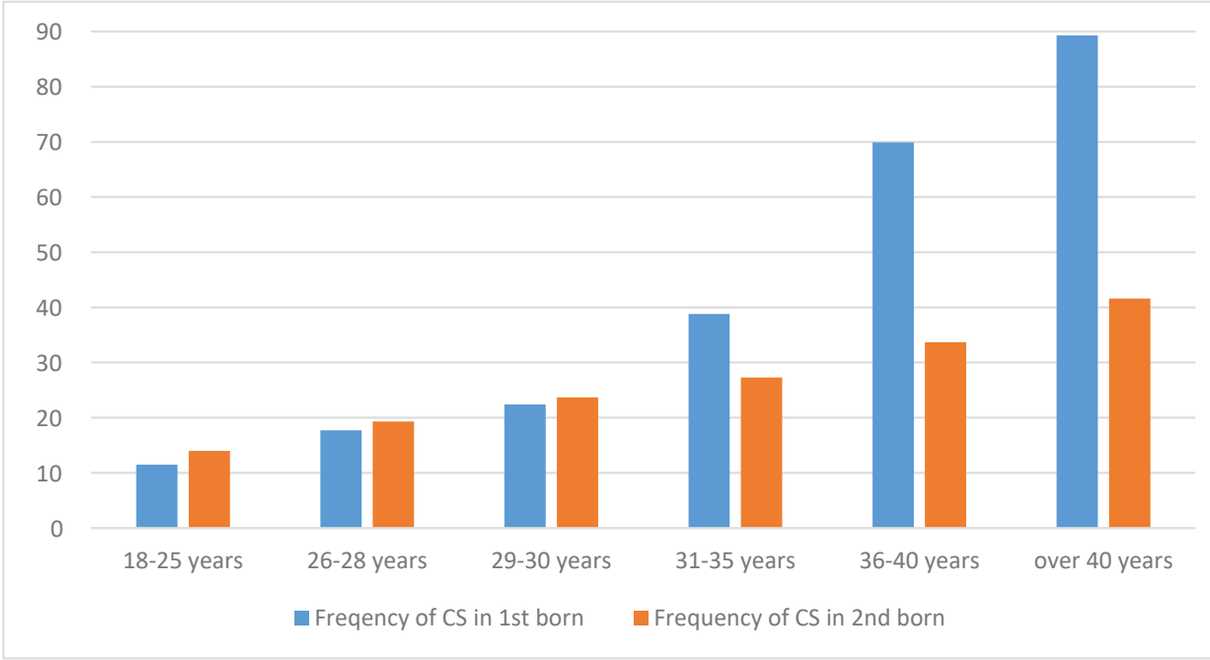


Figure 7. ***Frequency of CS in primi – and multiparous women.***

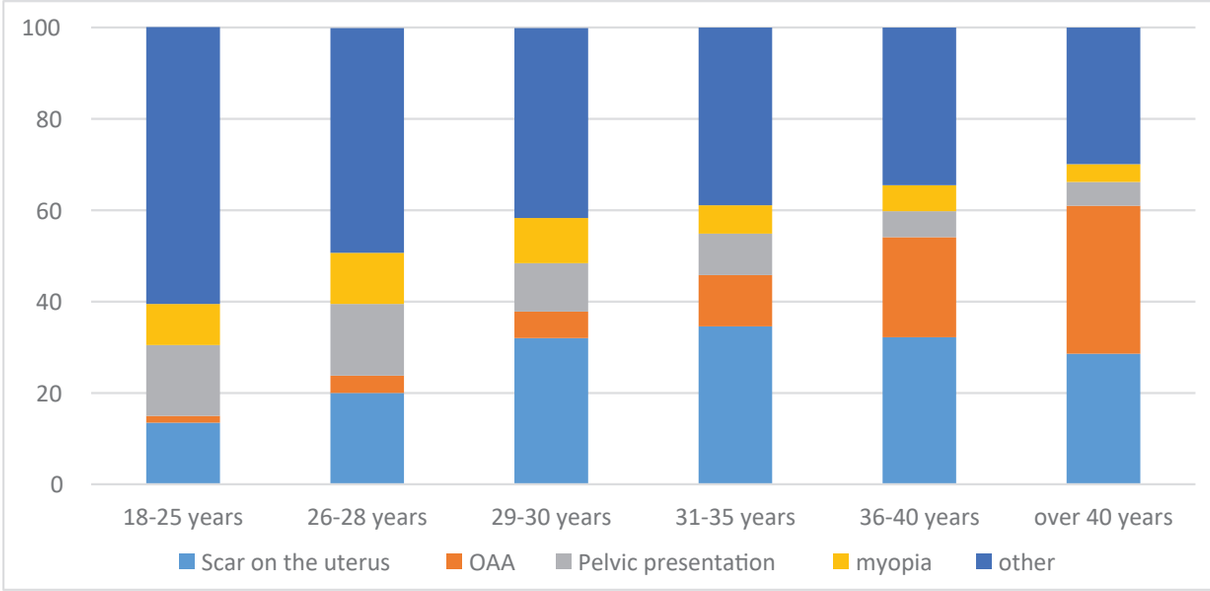


Figure 8. ***Leading indications for CS in various age groups.***

When studying the structure of indications for Caesarean section, we found that with increasing patient age, the proportion of such indications for CS as extragenital diseases, unfavorable perinatal outcome in the anamnesis, prolonged infertility, repeated IVF attempts, etc. sharply increases (Fig. 8).

Thus, to conduct an objective analysis of the dependence of the peculiarities of pregnancy and childbirth, as well as fertility, on the patients' age, we should use the gradations of age groups proposed by us (18-25, 26-28, 29-30, 31-35, 36-40 years, above 40 years), unlike the "traditional" division into two age groups (younger and older than 30 years).

The duration of labor in multiparous patients in all age groups we have identified does not depend on the interbirth interval, and, therefore, the long interval between deliveries should not serve as a basis for expanding the indications for a planned Caesarean section.

REFERENCES

to the article by L.G. Sichinava, O.B. Panina, E.P. Ozimkovskoy, and T. A. Kolbaya, “The course of pregnancy and childbirth in women of different age groups”.

1. Baev OR, Belousova VS Anomalies of labor activity in primiparous women above 30. *Gynecology, Obstetrics and Perinatology*. 2005; 4(1): 5-10.
2. Belousova VS. The course of pregnancy, childbirth and perinatal outcomes in primiparous women over 30 years old. Synopsis. Candidate of Medical Sciences; 2004: 23.
3. Kamaev IA, Pozdeyeva TV, Samartseva IY. Problems of reproductive health in teenage students. *Healthcare RF*. 2003; (1): 34-35.
4. Savelyeva GM, Kurtzer MA, Shalina RI. The role of intranatal fetus protection in improving perinatal outcomes. *Obstetrics and Gynecology*. 2000; (5): 3-8.
5. Strizhakov AN, Timokhina TF, Baev OR. Fetoplacental insufficiency: pathogenesis, diagnosis, treatment. *Gynecology, Obstetrics and Perinatology*. 2003; 2(2): 53-63.
6. Al-Turki HA, Abu-Heija AT, Al-Sibai MH. The outcome of pregnancy in elderly primigravidas. *Saudi Med J*. 2003; 24: 1230-1233.
7. Carolan M. Late motherhood: the experience of parturition for first time mothers aged over 35 years. *Aust J Midwifery*. 2003; 16: 17-20.
8. Ebclab U, Vilpa T. Pregnancy in women over forty. *Ann Chir Gynaecol Suppl*. 1994; 208: 68-71.
9. Heimann F, Messerer D, Baltzer J. Schwangerschaft, Geburtverlauf und Fetal Outcome bei Frauen über 40 Jahre. *Geb. u. Frauenh.* 1993; (6): 411-415.
10. Kozinszky Z, Orvos H, Katona M, et al. Perinatal outcome of induced and spontaneous pregnancies of primiparous women aged 35 or over. *Int J Gynecol Obstet*. 2002; 76(1): 23-26.
11. Mahfouz AA, El-Said MM, al-Erian RA, Hamid AM. Teenage pregnancy: are teenagers a high risk group? *Eur J Obstet Gynecol Reprod Biol*. 1995; 59(1): 17-20.
12. Miletic T, Aberle N, Mikulandra F, et al. Perinatal outcome of pregnancies in women aged 40 and over. *Coll Antropol*. 2002; 26(1): 251-255.
13. Sibai B, Ewell M, Levine RJ, et al. Risk factors associated with preeclampsia in healthy nulliparous women. *Am J Obstet Gynecol*. 1997; 177(5): 1090-1099.

CHROMOSOMAL ANOMALIES IN COUPLES WITH RECURRENT PREGNANCY LOSS

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SUMMARY

This study explores the landscape of chromosomal anomalies in couples with recurrent pregnancy loss (RPL) who have not previously delivered abnormal fetuses. From 2014 to 2021, we analyzed 122 couples who had experienced more than two first-trimester miscarriages. After excluding common causes of RPL, we conducted a cytogenetic analysis using G-banding. The results revealed chromosomal anomalies in 8.2% of cases, including balanced reciprocal translocations in 4 men and two women, Robertsonian translocations in 2 men, and subfertility in 3 men. Additionally, one woman had a pericentric inversion of chromosome 9, and another had a mosaic karyotype 46, XX/47, XXX. The introduction of genetic counseling led to two successful pregnancies with normal karyotypes. These findings underscore the potential of karyotyping to identify genetic causes and inform reproductive planning, empowering couples to make informed decisions about their future pregnancies.

Keywords: Recurrent pregnancy loss, chromosomal anomalies, balanced translocation, Robertsonian translocation, cytogenetic analysis, genetic counseling, karyotyping

INTRODUCTION

Genetic factors are the most frequent causes of spontaneous abortions (SA). Numerical chromosomal anomalies, such as aneuploidy or polyploidy, are detected in 50-80% of first-trimester miscarriages. The detection rate depends on the investigation methods used (e.g., FISH, CGH microarray), the composition of the groups studied (such as women of advanced age), and the specifics of family or obstetric history.[1,2] Most chromosomal abnormalities that cause miscarriage have random character. First-trimester SA expresses most (90%). However, these abnormalities might be associated with RPL.[3,4] According to different data, the frequency of chromosomal ab-

normalities in couples with RPL is 2-6%. [1,5,6] Translocation in one of the partners is a common and confirmed cause of recurrent miscarriage[7,8]. Prevalence of balanced translocations is higher in females than in males and higher in couples with a family history of stillborn or abnormal liveborn and, according to some authors, in subfertile men.[1,3,9,10]

Based on a meta-analysis of 79 studies, Tharapel A.T. et al. revealed that among couples with RPL, the structure of identified chromosomal abnormalities is as follows: either partner of couples with RPL has balanced reciprocal translocation in 50%, Robertsonian translocation in 24%, sex chromosome mosaicism in 12%, and in other cases, inversions and different sporadic chromosomal abnormalities were observed.[11]

The presence of a balanced chromosomal rearrangement in one partner can result in an unbalanced translocation in offspring. Phenotypic consequences (abort uses or abnormal liveborn) depend on the specific duplicated or deficient chromosomal segments. [1,2,5]

Translocations do not correlate with the age of mothers and the number of previous miscarriages. [1,6,12]

The theoretical risk of transmission of balanced translocations to offspring in unbalanced form is considerably higher than the empirical risk, which might be explained by the lethality of many segregant products.[1,5,6,8]

Different chromosomal aneuploidies may be expressed in translocation cases due to interchromosomal effects.[1,13] In first-trimester abortions, recurrent aneuploidy occurs more often than expected by chance, which might be tied to the mother's age and also to germ cell mosaicism.[1]

According to the last period data, in cases of structural abnormalities of chromosomes, IVF accompanied by PGD decreases the risk of spontaneous abortions but also decreases the chance of live birth compared to spontaneous pregnancy. In spontaneous pregnancies, considering concomitant factors, the live birth chance is up to 70%.[2,5,12]

There are no standard views on the necessity of karyotyping concepts or whether the karyotyping of couples with RPL (RCOG, ASRM, ECHRE protocols) is economically justified.[14,15]

Some experts recommend karyotyping couples with RPL if there is no information on the Karyotype of conceptuses.[15, 16]

Detection of frequency and types of chromosomal anomalies in couples with I trimester RPL without the history of delivery with the abnormal fetus.

METHODS

One hundred twenty-two couples with > 2 first trimester miscarriages were involved in a prospective observational study in 2014-21 based on the Center for Reproductive Medicine "Universe" and the Georgian Centre of Prenatal Diagnostics.

The mean age of women was 30,3+2, and the mean age of men – was 32.1+3.

In all cases, family history and obstetric anamnesis were collected and analyzed.

Common causes of RPL—anatomic (congenital and/or acquired), hormonal (luteal insufficiency, diabetes, thyroid dysfunction, PCOS, hyperprolactinemia, etc.), and immunological (APS)—were excluded for all couples;

All couples have undergone cytogenetic investigation. The Karyotype was detected in peripheral blood lymphocyte cultures (G-banding).

Ethical considerations: Written informed consent was obtained from all participants before their inclusion in the study.

RESULTS

Personal or family history of pregnancy and delivery of a fetus with congenital anomalies or child with mental retardation was not detected in any of the cases;

The Karyotype of previous concepts was not investigated in any of the cases;

The mean number of previous miscarriages in the standard group of RPL was 3,15, and in the couples with chromosomal anomalies – 2,9; Chromosomal anomalies in one partner were revealed in 10 cases (8.2%) (Table 1) Balanced reciprocal translocations were detected in 4 men and two women (Fig. 1), Robertsonian translocation – in 2 men, and three from 6 men with translocations (2 Robertsonian and one reciprocal) were subfertile (oligozoospermia); The total frequency of balanced translocations was 6,6%[8]; One woman had a pericentric inversion of chromosome 9, and one woman – had mosaic karyotype 46, XX/47, XXX.

Table 1. ***Type of Chromosomal Anomalies and Reproductive disorders in couples with RPL***

N	Karyotype	Numbers of first trimester miscarriages	Other reproductive disorders
1	46, XX, t (2;13) (p14;q32)	2	
2	46, XX, t (5;16) (p12;q22)	2	
3	46, XY, t (2;9) (p22;p24)	2	
4	46, XY, t (18;21) (q22;q21)	3	
5	46, XY, t (10;18) (q11,2; q2,1)	3	subfertility (olygozoospermia)
6	46, XY, t (6;22) (p21.3;q13.3)	4	
7	45, XY, rob (13;15) (q10;q10)	4	subfertility (olygozoospermia)
8	45, XY, rob (13;14) (q10;q10)	3	subfertility (olygozoospermia)
9	46, XX, inv (9) (p11;q12)	3	
10	46, XX / 47XXX (18/32)	3	

Figure 1.

Couple with 2 SA Woman 21y old, Karyotype 46, XX, t (2;13) (p14;q32)

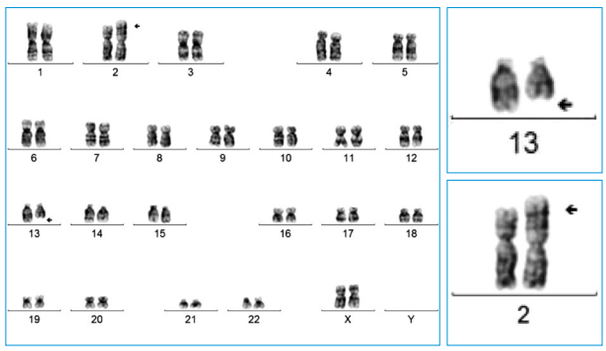
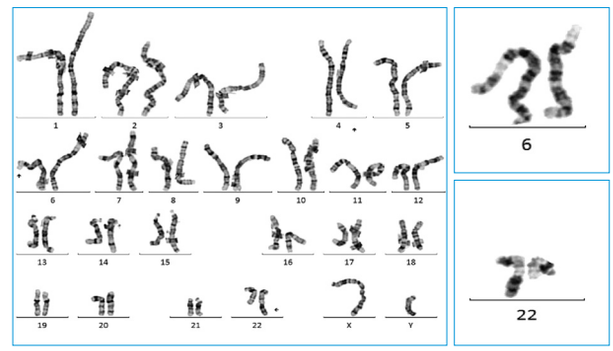


Figure 2.

Couple with 4 SA Male partner 30 y old, Karyotype 46, XY, t (6;22) (p21.3;q13.3)



Pericentric inversion of chromosome 9 was revealed in 1 woman with a history of 3 previous I trimester spontaneous abortions, karyotype – 46, XX, inv^{9}(p11; q12); Pericentric inversion of chromosome 9 is considered as a variant of normal Karyotype with incidence 1-3% of the general population.[17] This inversion does not correlate with abnormal phenotypes, but in the literature exist, conflicting views regarding the association of this variant with such clinical conditions as infertility, RPL, and stillbirth.[17,18,19]

A mosaic karyotype 46, XX/47, XXX (37/63) was found in 1 woman with a history of 3 previous I-trimester spontaneous abortions. It is important to note that sex chromosome polysomy is a scary condition, occurring in only 0.05% of spontaneous abortuses, and it is not incompatible with life.[1]

DISCUSSION

The causative relationship of pericentric inversion of chromosome 9 and X chromosome polysomy with RPL needs further investigation. Genetic counseling was conducted for all couples with detected chromosomal anomalies, informing them of their risks and reproductive opportunities, including IVF with PGD, spontaneous pregnancy with or without CVS or amniocentesis, gamete donation, and child adoption.

Following genetic counseling, two women achieved spontaneous pregnancies. One 24-year-old woman, whose 26-year-old husband had a reciprocal translocation 46, XY,t$^{6;22}$(p21.3;q13.3) and a history of four previous first-trimester spontaneous abortions, became pregnant and received intensive prenatal care and psychological support. Noninvasive prenatal genetic screening results were expected, and the pregnancy ended with the timely physiological delivery of a phenotypically usual girl with a karyotype of 46, XX. Another 39-year-old woman with a history of two previous first-trimester spontaneous abortions, who had a reciprocal translocation 46, XX-,t$^{5;16}$(p12;q22), also achieved spontaneous pregnancy. At 18 weeks, fetal balanced translocation (similar to the maternal) was detected by amniocentesis. The pregnancy was maintained and ended with the physiological delivery of a phenotypically normal fetus.

Revealing the natural causes of RPL by karyotyping couples might benefit these couples and the experts managing their cases. Our results indicate that karyotyping couples with RPL without a history of delivering abnormal fetuses is reasonable, as chromosomal anomalies among them are not rare.[8,2] Balanced chromosomal rearrangement in one partner can result in an unbalanced translocation in offspring, and phenotypic consequences (abortions or abnormal live births) depend on the specific duplicated or deficient chromosomal segments.[1,2] These chromosomal disorders can often be clinically revealed mainly by spontaneous abortions.[3,13]

CONCLUSION

In couples with RPL and without a history of delivery with the abnormal fetus, when the chromosomal status of previous miscarriages is unknown, considerable frequency of balanced structural chromosomal anomalies (with prevalence in male partners- 6/2) indicates on the reasonability of karyotyping of such couples, especially when the male partner is subfertile.

REFERENCES

1. Simpson JL, Elias S, Martin AO. Genetics of spontaneous abortions. In: Recurrent Pregnancy Loss: Causes, Controversies and Treatment. CRC Press; 2015: 29-42.

2. Rabinowitz M, Ryan A, Gemelos G, Hill M, Baner J, Cinnioglu C, Banjevic M, et al. Origins and rates of aneuploidy in human blastomeres. *Fertil Steril.* 2012; 97(2): 395-401.
3. Elhady GM, Kholeif S, Nazmy N. Chromosomal aberrations in 224 couples with recurrent pregnancy loss. *J Hum Reprod Sci.* 2020; 13(4): 340-348.
4. Suzumori N, Sugiura-Ogasawara M. Genetic factors as a cause of miscarriage. *Curr Med Chem.* 2010; 17(29): 3431-3437.
5. Li S, Chen M, Zheng PS. Analysis of parental abnormal chromosomal Karyotype and subsequent live births in Chinese couples with recurrent pregnancy loss. *Sci Rep.* 2021; 11: 20298.
6. Ashalatha PR, Priya N, Manju M, Shyja. Recurrent pregnancy loss – chromosomal anomalies in couples. *Int J Biomed Res.* 2021; 12(2): e5576.
7. Simpson JL, Elias S, Martin AO. Parental chromosomal rearrangement associated with repetitive spontaneous abortion. *Fertil Steril.* 1981; 36(5): 584-590.
8. Goddijn M, Joosten JHK, Knegt AC, van der Veen F, Franssen MTT, et al. Clinical relevance of diagnosing structural chromosomal abnormalities in couples with repeated miscarriage. *Hum Reprod.* 2004; 19: 1013-1017.
9. Simpson JL, Elias S, Martin AO. Translocations are infrequent among couples having repeated spontaneous abortions but no other abnormal pregnancies. *Fertil Steril.* 1989; 51(5): 811-814.
10. Rajangan S, Tilak P, Aruna N, Devi R, et al. Karyotyping and counseling in bad obstetric history and infertility. I*ran J Reprod Med.* 2007; 5(1): 7-12.
11. Tharapel AT, Tharapel SA, Bannerman RN. RPL and parental chromosome abnormalities: a review. *Br J Obstet Gynaecol.* 1985; 92(9): 899-914.
12. Kochhar PK, Ghosh P. Reproductive outcomes of couples with recurrent miscarriage and balanced chromosomal abnormalities. *J Obstet Gynaecol Res.* 2013; 39: 113-120.
13. Alibakhshi R, Nejati P, Hamani S, MirAhadi N, Jalilian N. Cytogenetic analysis of 570 couples with recurrent pregnancy loss: reporting 11 years of experience. *J Hum Reprod Sci.* 2020; 13(3): 216-220.
14. Carp H. Should fetal karyotyping be performed in RPL? Yes. In: RPL Causes, Controversies and Treatment. CRC Press; 2015: 43-49.
15. Borochowitz Z. Should fetal karyotyping be performed in RPL? No. In: RPL Causes, Controversies and Treatment. CRC Press; 2015: 49-55.
16. Kuttech WH, Raymond WK, Brezina PR. A new algorithm for evaluation and treatment of RPL. In: RPL Causes, Controversies and Treatment. CRC Press; 2015: 389-400.
17. Mierla D, Stoian V. Association of pericentric inversion of chromosome 9 and infertility in Romanian population. *Maedica* (Buchar). 2012; 7(1): 25-29.
18. Simpson J, Bombard A. Spontaneous abortion. In: Edmunds KB, ed. Spontaneous Abortion. Oxford: Blackwell; 1987: 51-76.
19. Nonaka T, Takahashi M, Nonaka C, Enomoto T, Takakuwa K. The analysis of chromosomal abnormalities in patients with recurrent pregnancy loss, focusing on the prognosis of patients with inversion of chromosomes. *Reprod Med Biol.* 2019; 18: 296-301.

RISKS OF GONADAL MALIGNANCY AND REPRODUCTIVE PROGNOSIS IN INDIVIDUALS WITH CONGENITAL SEX DEVELOPMENT DISORDERS

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SUMMARY

The risk of gonadal malignancy varies according to the type of sex development disorder. It depends on the presence of the Y chromosome in the karyotype and the location of the gonads.

Aim of the study: Assessment of the risks of gonadal malignancy and reproductive prognosis in female phenotype patients with congenital disorders of sex development and Y chromosome in karyotype.

Materials and methods: 48 patients with female phenotype and congenital disorders of sex development, with detected Y chromosome in the karyotype (46, XY and 45,X/46,XY) were examined. All patients underwent clinical, gynecological, hormonal, and ultrasound examinations. In 32 cases, gonadectomy was performed. A Histomorphological study of excised gonads was carried out. Based on conducted examinations, a complete form of 46,XY gonadal dysgenesis (Swyer syndrome) was identified in 3 cases, in 2 cases – a partial form of gonadal dysgenesis with a background of the Turner syndrome phenotype. The complete form of androgen insensitivity syndrome (CAIS) was diagnosed in 33 cases, and a partial form of androgen insensitivity syndrome (PAIS) in patients with female phenotype – in 8 cases. In 2 cases, an ovotesticular disorder was established.

Results: Patients with complete and partial forms of gonadal dysgenesis with intra-abdominal localization of gonads, taking into account the high risk of malignancy, underwent gonadectomy immediately after diagnosis, regardless of age, to prevent malignancy. Subsequently, they were prescribed hormone therapy with estrogens and estrogen-gestagens. Despite the pessimistic reproductive prognosis in 2 cases, pregnancy and the birth of a healthy child was achieved with egg donation.

Keywords: Gonadal malignancy, Y chromosome, sex development disorder, gonadectomy, androgen insensitivity syndrome, reproductive prognosis, Swyer syndrome

Considering the low risk of gonadal malignancy in patients with CAIS, 25 patients underwent intra-abdominal testicular excision after the end of puberty and were prescribed monotherapy with estrogens. In one case, in a patient with CAIS at the age of 18, a seminoma of the intra-abdominal gonad has been detected, which is extremely rare. A 41-year-old patient with CAIS gonadoblastoma was diagnosed by histomorphological examination in the inguinal located gonad after gonadectomy.

In adolescent patients with female phenotype and PAIS with a high risk of gonadal malignancy, gonadectomy was performed immediately after diagnosis, regardless of gonadal localization, to prevent malignancy and to stop the masculinization effect. Subsequently, they were prescribed monotherapy with estrogens. Reproductive prognosis for patients with CAIS and PAIS is pessimistic, although it is possible to have a child using a male partner's sperm in a donor-surrogacy program.

Despite a low risk of malignancy in female phenotype patients with ovotesticular disorder and 46,XY karyotype underwent intra-abdominal gonadectomy as neither ovotestis nor contralateral testis contained structures usable for reproduction. Patient management and reproductive prognosis are similar to the CAIS in such cases.

Conclusions: Timely and correct diagnosis and management of congenital sex development disorders is the most reliable approach to gonadal malignancy prevention. After that, it is possible to select adequate methods of assisted reproductive technologies, which is reflected in the improvement of the quality of life of such individuals.

Timely diagnosis and optimal management of congenital sex development disorders (SDD) are essential not only for minimizing main complaints related to the reproductive system of patients (delayed sex development, amenorrhea, inadequate sexual development, infertility, etc.) but also for preventing such long-term complications as osteoporosis, cardiovascular diseases and in some cases increased risk gonadal malignancy.[1-7]

The risk of gonadal malignancy differs by type of SDD and depends on the presence of the Y chromosome in the karyotype and gonadal localization.[3,7,8]

Risks of gonadal malignancy by diagnoses and recommendations (Tab. 1)

Risk group	Disease	Risk of malignancy (%)	Recommendation
High	Complete and partial forms of gonadal dysgenesis (+Y), with intra-abdominal localization of gonads	15-35	Gonadectomy immediately after diagnosis
	Partial forms of androgen insensitivity syndrome with non-scrotal gonads	50	Gonadectomy immediately after diagnosis
Average	Turner syndrome (+Y)	12	Gonadectomy immediately after diagnosis
	Partial forms of androgen insensitivity syndrome with scrotal gonads	Unknown	Biopsy and radiological investigation
Low	Complete form of androgen insensitivity syndrome	2	Biopsy and radiological investigation
	Ovotesticular SDD	3	Excision of testicular tissue?
	Turner syndrome (-Y)	1	No

AIM OF THE STUDY

Assessment of the risks of gonadal malignancy and reproductive prognosis in female phenotype patients with congenital SDD and Y chromosome in the karyotype.

MATERIALS AND METHODS

48 patients with female phenotypes and congenital sex development disorders were examined. A Y chromosome was detected in the karyotype (46, XY and 45, X/46, XY).

All patients underwent clinical, gynecological, hormonal, and ultrasound examinations. In 32 cases, gonadectomy was performed. Histomorphological study of excised gonads was carried out. Based on conducted examinations, a complete form of 46, XY gonadal dysgenesis (Swyer syndrome) was identified in 3 cases, in two instances – a partial form of gonadal dysgenesis with a background of the Turner syndrome phenotype. The complete form of androgen insensitivity syndrome (CAIS) was diagnosed in 33 cases, and a partial form of androgen insensitivity syndrome (PAIS) in patients with female phenotype – in 8 cases. In 2 cases, an ovotesticular disorder was established.

ETHICAL CONSIDERATIONS

All the adult participants and parents of adolescent individuals signed a written consent form.

RESULTS AND DISCUSSION

Among the patients with SDD under our observation, three were diagnosed with Swyer syndrome (a complete form of gonadal dysgenesis). The patients' ages corresponded to 15, 19, and 24.

All three patients were of the female phenotype and were of average height. No somatic anomalies or visceropathies were observed. In all cases, clinical and hormonal features characteristic of hypogonadotropic hypogonadism were established. The karyotype in all three cases was 46, XY in peripheral blood lymphocyte culture.

Considering that the risk of gonadal malignancy in the complete form of 46, XY gonadal dysgenesis is high (15-35%), all three patients underwent gonadectomy immediately after the diagnosis.[9,10,11] By histomorphological study, streak gonads were represented by connective tissue. In one case, epithelial cells of Leydig cell type were detected. After gonadectomy, patients were prescribed hormone therapy with estrogens and estrogen-gestagens, as well as vitamin D and calcium preparations. As a result of the treatment, patients showed secondary sexual characteristics and an enlarged uterus. After treatment, two patients became pregnant using egg donation, carried the pregnancy to term, and gave birth to a healthy child. One patient chose to adopt a child.

Among the patients examined, two adolescents (15 and 16 years old) had Turner's phenotype (height 138 and 141 cm), visceropathies were detected in no case, both patients had clinical and laboratory characteristics of hypergonadotropic hypogonadism. They had hairiness on the pubic area, and the clitoris was not sharply hyperplastic. Ultrasonographic examination revealed a streak uterus and unilateral streak gonad; on the other side, a gonad without follicles. In both cases, the karyotype was mosaic 45, X /46, and XY.

A diagnosis of mixed, partial gonadal dysgenesis was made. In such cases, taking into account the increased risk of gonadal malignancy and current recommendations, laparoscopic bilateral gonadectomy was performed.[3,9,12] By histomorphological study, streak gonads were represented by connective tissue structures, gonads on the other side – with dysgenetic testicles. Clitorectomy was performed in both cases. After surgery, hormone therapy with estrogens and estrogen-gestagens, vitamin D, and calcium preparations were prescribed. On the background of hormone therapy, mamae growth was noted, and the uterus also grew. One patient tried to become pregnant through in vitro fertilization using an egg donation program after becoming sexually active, but two attempts were unsuccessful.

In both complete and partial forms of gonadal dysgenesis, taking into account the high risk of gonadal malignancy, gonadectomy is performed immediately after the diagnosis is made, and therefore the reproductive prognosis is pessimistic. However, in such cases, it is possible to achieve pregnancy and have a child using egg donation programs.[7,13]

Therefore, it is essential to diagnose those forms of SDD in time that are characterized by a high risk of gonadal malignancy in time so that a gonadectomy can be performed to prevent malignancy and hormone replacement therapy can be performed after the gonadectomy.

33 patients (12-28 years old) were diagnosed with a complete form of androgen insensitivity syndrome (CAIS), and eight patients (14-16 years old) with female phenotype were diagnosed with a partial form of androgen insensitivity syndrome (PAIS).

Patients with CAIS had female phenotype, female passport gender and psychosexual orientation, well-developed mamae, sparse pubic hair, female-type external genitalia without clitoromegaly, and short, blind-ending vagina.

Ultrasonography did not detect the uterus. Gonads of different localizations were detected (localized intra-abdominally, inguinally, or at the thickness of the labia majora).

In all cases, hormonal parameters were typical for males, and the karyotype was consistent with the 46, XY male norm. Considering that the risk of gonadal malignancy in CAIS cases is low, patients with intra-abdominal and inguinal localizations underwent gonadectomy after puberty was completed. Besides, it is known that the risk of malignancy increases with age.[8,14]

One of our patients with CAIS, at the age of 41, was diagnosed with gonadoblastoma by histological study after inguinal testis resection.

According to literature data,[1] some patients with CAIS report a decrease in libido after gonadectomy. No changes in sexual function were detected after gonadectomy in patients under our observation. One patient postponed orchiectomy because she had good sexual penile-vaginal contact with a man, with frequent orgasms, and feared that orchiectomy would negatively affect her sexual life.

All patients with CAIS after gonadectomy were prescribed monotherapy with estrogens, vitamin D, and calcium preparations. Despite the low risk of gonadal malignancy in CAIS cases, one 18-year-old adolescent patient with CAIS was diagnosed with seminoma in intra-abdominally localized left testis, which is considered a rare case.[15]

Thus, despite the low risk of gonadal malignancy in patients with CAIS, considering rare cases, it is advisable to monitor intra-abdominally localized testes from the puberty period. 76

All patients with PAIS[8], who were assessed as girls with female psychosexual disposition, were referred to us due to revealing the signs of masculinization during adolescence. As soon as the diagnosis was made, regardless of the location of gonads, all our adolescent individuals underwent bilateral orchiectomy according to the protocol to stop the progression of masculinization and considering that in the cases of PAIS, the risk of testicular malignancy is increased (50%).[1,8] These individuals underwent feminizing genitoplasty and were prescribed estrogen replacement therapy. After 1-year follow-up, an increase in mamme, change in tone of voice, and improvement in mood were found in these individuals.

Reproductive prognosis in both CAIS and PAIS patients assessed as girls is pessimistic. As a way to have a child, these patients can consider joining a donation-surrogacy program using the male partner's sperm or adopting a child.

Among our patients with SDD 46, XY ovotesticular disorder was detected in two patients (17 and 35 years old) with female phenotype. The diagnosis was confirmed by laparoscopic biopsy and histomorphological examination of gonads after gonadectomy through the establishment of unilateral ovotestis. In both cases, hypoplastic testicular tissue with clearly manifested fibrosis was detected in the gonad on the other side. A 35-year-old patient with 46, XY ovotesticular SDD was married to a man and referred us due to infertility.

Although the risk of ovotestis malignancy is relatively low (3%),[3,9] in these patients with 46, XY karyotype structurally incomplete uterus in the form of Müllerian derivatives and the presence of dysgenetic testicle on one side and hypoplastic testicular tissue in the ovotesticle on the other side due to absence of reproductive potential, it was advisable to perform gonadectomy after obtaining patient's informed consent. After surgery, patients were prescribed hormone replacement therapy with estrogen.

It is impossible to consider ovotesticular disorder as a single syndrome because there is no typical phenotype or karyotype characteristic for it. Thus, the presence of ovotestis is a determinant in diagnosis, which is established only by histomorphological study. Phenotypes may differ: feminine, ambisexual, or masculine.[3,7,16]

Reproductive prognosis: most often pessimistic due to incomplete development of the Müllerian ducts (uterus, fallopian tubes, upper two-thirds of the vagina) and gonadal tissue, as well as the need to perform gonadectomy. However, taking into account the development of modern reproductive technologies in the recent period, in individual cases, it is possible to consider the expediency of cryopreservation of reproductive materials before gonadectomy. It is known that in ovotesticular disorders, ovulation is observed in 50% of cases. Pregnancy and even delivery have been described in such patients, suggesting that there may be normal development of both female-type external and internal genitalia, even in the cases of 46, XY ovotesticular disorder.[17]

The literature describes 12 cases of spontaneous pregnancy in patients with 46, XX ovotesticular SDD with a female phenotype.[16] Only one case of fertility in a 46 XY patient has been described. In all those cases, surgical excision of testicular tissue was performed before pregnancy. In such patients, excision of androgen-producing testicular tissue may improve ovulation.[17]

One of our patients with the ovotesticular disorder and female phenotype with 46, XX karyotype was diagnosed by diagnostic laparoscopy and gonadal biopsy, having an ovary on one side

and 77 ovotestes on the other side with signs of ovulation. Taking into account the patient's desire, it was planned to cryopreserve the eggs and subsequently use them in the surrogacy program, as the patient did not have a uterus, and only Müllerian derivatives were presented. Müllerian derivatives were excised during surgery.

Thus, in the cases of ovotesticular disorders, the reproductive prognosis is sharply different – from pessimistic to favorable and a solution to the issue of gonadectomy requires an individual approach, taking into account reproductive potential and the anatomy of the genitals.

CONCLUSION

- In patients with complete and partial forms of gonadal dysgenesis, in the presence of Y chromosome in the karyotype, taking into account the high risk of gonadal malignancy, gonadectomy should be performed immediately after diagnosis. After gonadectomy, it is possible to achieve pregnancy using egg donation on the background of estrogen-gestagen replacement hormone therapy.
- Despite the low risk of gonadal malignancy in complete forms of androgen insensitivity syndrome, it is advisable to monitor the gonads from puberty and, based on this, to plan gonadectomy in the postpubertal period and then monotherapy with estrogens. Reproductive prognosis is pessimistic, although it is possible to have a child using a male partner's sperm through surrogacy-donation programs.
- In partial forms of androgen insensitivity syndrome, gonadectomy in patients with a female passport gender and psychosexual disposition should be performed immediately after the diagnosis is made, considering the high risk of malignancy and to prevent the progression of masculinization in any location of the gonads. Feminizing genitoplastic surgery and estrogen replacement therapy are necessary to achieve feminization, improve quality of life, and prevent long-term complications. Reproductive prognosis is similar to complete forms of androgen insensitivity syndrome.
- Considering the low risk of ovotesticular malignancy in case of ovotesticular disorders, it is possible to preserve it and, if possible, cryopreserve eggs for later use in assisted reproductive technology (ART) programs. Excision of the testicle on the other side increases the chances of ovulation in the ovotestis. Comprehensive clinical polymorphism of patients with ovotesticular disorders requires an individualized approach in selecting the type of assisted reproductive technology, taking into account the anatomy of the gonads and internal genital organs.

REFERENCES

1. Ovidiu B, Marcu DR, Dan LD, Mischianu DL, Poiana CC, Camelia C, et al. The challenges of androgen insensitivity syndrome. *Arch Med Sci.* 2022; 18: 881-889.
2. Taylor H, Pal L, Seli E. Speroff's Clinical Gynecologic Endocrinology and Infertility. 9th ed. *Wolters Kluwer;* 2020.
3. Hughes IA, Houk C, Ahmed SF, Lee PA; LWPES Consensus Group; ESPE Consensus Group. Consensus statement on management of intersex disorders. *Arch Dis Child.* 2006; 91: 554-563.

4. Elsheikh M, Dunger D, Conway G, Wass J. Turner's syndrome in adulthood. *Endocr Rev.* 2002; 23(1): 120-140.
5. Salomskiene L, Andriuskeviciute I, Jurkeniene L, Sinkus A. The Lithuanian collection of 224 Turner syndrome patients. *Eur J Hum Genet*. 2005; 13(1): 147.
6. Gulia C, Baldassarra S, Zangari A, et al. Androgen insensitivity syndrome. *Eur Rev Med Pharmacol Sci.* 2018; 22: 3873-3887.
7. Consortium on the Management of Disorders of Sex Development. Clinical guidelines for the management of disorders of sex development in childhood. *Intersex Society of North America*; 2006.
8. Cools M, Looijenga LH, Wolffenbuttel KP, T'Sjoen G. Managing the risk of germ cell tumourigenesis in disorders of sex development individuals. *Endocr Dev.* 2014; 27: 185-196.
9. Cools M, Drop S, Wolffenbuttel K, Oosterhuis J, Looijenga L. Germ cell tumors in the intersex gonad: Old paths, new directions, moving frontiers. *Endocr Rev.* 2006; 27(5): 468-484.
10. Cools M, Stoop H, Kersemaekers AF, et al. Gonadoblastoma arising in undifferentiated gonadal tissue within dysgenetic gonads. *J Clin Endocrinol Metab.* 2006; 91(6): 2404-2413.
11. Hoepffner W, Horn LC, Simon E, et al. Gonadoblastomas in 5 patients with 46, XY gonadal dysgenesis. *Exp Clin Endocrinol Diabetes*. 2005; 113(4): 231-235.
12. El Moussaif N, Haddad NE, Iraqi N, Gaouzi A. 45, X/46, XY mosaicism: Report of five cases and clinical review. *Ann Endocrinol* (Paris). 2011; 72(3): 239-243. doi: 10.1016/j.ando.2011.04.004
13. Wolffenbuttel KP, Hersmus R, Stoop H, et al. Gonadal dysgenesis in disorders of sex development: Diagnosis and surgical management. *J Pediatr Urol.* 2016; 12: 411-416.
14. Batista RL, Costa EMF, Rodrigues ADS, et al. Androgen insensitivity syndrome: A review. *Arch Endocrinol Metab.* 2018; 62: 227-235.
15. Chen C, Chern S, Chen B, Wang W, Hwo Y. Hamartoma in pubertal patient with complete androgen insensitivity syndrome and R831X mutation of the androgen receptor gene. *Fertil Steril.* 2000; 74(1): 182-183.
16. Schultz BA, Roberts S, Rodgers A, Ataya K. Pregnancy in true hermaphrodites and all male offspring to date. *Obstet Gynecol.* 2009; 113: 534-536.
17. Dumic M, Lin-Su K, Leibel N, et al. Report of fertility in a woman with predominantly 46, XY karyotype in a family with multiple disorders of sexual development. *J Clin Endocrinol Metab.* 2008; 93(1): 182-189.

THYROID FUNCTION AND FERTILITY IN WOMEN

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SUMMARY

Thyroid hormones, by affecting prolactin and sex hormone-binding globulin levels and oocyte maturation, significantly affect reproductive function in women. Subclinical or overt hypothyroidism is most common, and hyperthyroidism is less common. Approximately 10% of women show immunological thyroid disorders with increased antibody levels. There is no conclusive data that fertility disorders accompany hyperthyroidism. Hypothyroidism in pregnancy is a factor in its risk, as therapy in the first trimester is proposed propylthiouracil. In contrast, thiamazole is recommended in the second and third trimesters due to its hepatotoxic effects. Breastfeeding should occur immediately after taking the drugs and 3 hours before the next feeding. Abnormalities in monthly bleeding and ovulation accompany hypothyroidism and are the most common cause of fertility and pregnancy disorders, especially when accompanied by elevated levels of anti-TPO antibodies. Medications used to treat hypothyroidism are not contraindicated for breastfeeding during pregnancy, and screening tests in the form of TSH, FT4, and anti-TPO determination are recommended for women planning pregnancy and who are pregnant.

Keywords: Thyroid, fertility, pregnancy, hyperthyroidism, hypothyroidism

INTRODUCTION

Thyroid hormones are essential for the proper development and differentiation of all human body cells and affect the female reproductive organ. Directly affecting the ovary and endometrium during the luteal phase and the trophoblast and placenta via the TH receptors (TRs): TRa1, TRa2, and TRb1.[1, 2, 3] Combined with FSH, triiodothyronine enhances granulose cell proliferation and inhibits granulose cell apoptosis by the protein kinase B pathway. Leukemia inhibitory factor (LIF) is involved in embryo implantation and expressed in the mid-secretory endometrium. TSH

significantly upregulates LIF expression in endometrial cell cultures, suggesting a potential role of TSH in the implantation process.[1, 2, 3] Thyroid hormones also regulate the secretion of prolactin and probably kisspeptin, which, in turn, affect pulsatile gonadotropin-releasing hormone secretion and hypothalamic-pituitary-gonadal axis function. Also, it was shown that thyroid hormones stimulating the synthesis of SHBG influence free sex hormone levels.[1, 2, 3] For that reason, we can expect that in women of reproductive age, thyroid dysfunction and/ or autoimmunity have independently been associated with adverse fertility and pregnancy outcomes in the case of spontaneous conception or after assisted reproductive technology (ART).[1, 2, 3] How pregnancy affects thyroid function: estrogen stimulates the synthesis of thyroxine-binding globulin (TBG), which causes a decrease in free thyroxine (FT4) and free triiodothyronine (FT3). In response, TSH secretion increases and FT4 and FT3 synthesis physiologically increases, and the size of the thyroid gland increases. At the same time, it is essential to remember the thyrotropic effect of βhCG. The concentration of hCG increases in the first trimester and peaks around the 10th week of pregnancy. It can cause TSH suppression, which is sometimes misinterpreted as hyperthyroidism. The thyrotropic effect of βhCG leads to pregnancy-related transient thyrotoxicosis in only about 2% of patients. At the same time, the peripheral metabolism of thyroid hormones is altered to maintain homeostasis and ensure the proper supply of maternal thyroid hormones to the fetus. When the availability of thyroxine decreases, the activity of 5'-deiodinase type 2 in the placenta increases to maintain a sufficiently high concentration of triiodothyronine in the placenta. In turn, 5'-deiodinase type 3, which converts thyroxine to the inactive reverse of triiodothyronine and inactivates triiodothyronine, protects against excess thyroxine. [1, 2, 3]

Hyperthyroidism, and Fertility Disorders, and Pregnancy

In hyperthyroidism, serum concentrations of sex hormone binding globulin (SHBG) and estradiol increase compared to pregnant women with normal thyroid function. This is associated with increased conversion of androgens to estradiol and estrone and increased secretion of LH. An increased risk of miscarriage accompanies this. It has been shown that lowered TSH in pregnant women is more often accompanied by elevated antibody levels compared to women with lowered TSH who are not pregnant.[6] Menstrual cycle abnormalities in women with hyperthyroidism occur in 65% compared to 17% of the healthy population.[7] Hypomenorrhea, polymenorrhea, oligomenorrhea, and hypermenorrhea are the most common menstrual abnormalities. Endometrial biopsy results indicate that most women with hyperthyroidism maintain ovulatory cycles.[8] In women with hyperthyroidism in pregnancy, we can expect several complications like Preeclampsia: OR 1. 78, Preterm birth: OR 1. 81 and Intensive care unit admission OR 2. 08 Superimposed preeclampsia OR 3. 64.[1, 2, 3] How to diagnose hyperthyroidism in pregnancy? Taking into account the previously described changes in TSH under the influence of HCG in the first trimester, the diagnosis of hyperthyroidism should include the determination of FT4 and FT3 levels because only elevated levels of these hormones with low TSH are the basis for initiating therapy for hyperthyroidism. Due to the passage of antithyroid drugs through the placenta, subclinical hyperthyroidism is not an indication for pharmacotherapy in pregnancy, as the risks of antithyroid medications outweigh the benefits. To determine the etiology of hyperthyroidism, measuring the level of antibodies to the receptor for TSH (TRAb) is recommended. Determination of antibody levels

is also recommended in cases of fetal tachycardia or the presence of goiter. The finding of more than fivefold elevation of TRAb levels is associated with the risk of fetal or neonatal hyperthyroidism. [1, 2] The most common cause of hyperthyroidism in pregnancy is Graves-Basedow disease. The differential diagnosis should include gestational thyrotoxicosis, gestational trophoblastic disease, subacute thyroiditis, and excess exogenous thyroid hormones. Rare causes include ovarian goiter and secondary hyperthyroidism due to a pituitary tumor. Treatment of hyperthyroidism in pregnancy is based solely on pharmacotherapy, excluding the "block and replace" method. Radioiodine treatment is contraindicated. In the first trimester, propylthiouracil (PTU) is used. Due to the hepatotoxic effects of PTU, therapy in the second and third trimesters is continued with thiamazole. The therapeutic goal is to maintain FT4 and FT3 levels in the upper range of reference values. Initially, it is recommended to monitor therapy every two weeks and after reaching euthyroidism every 2-4 weeks. Liver enzymes and white blood cell counts should be monitored during PTU treatment. Iodine prophylaxis is not contraindicated in hyperthyroidism in pregnant women. At a dose of methimazole ≤ 20 mg/day and PTU ≤ 300 mg/day, breastfeeding is not contraindicated. The drugs should be administered immediately after feeding, with an interval of 3 hours before the next feeding. [1, 2]

Hypothyroidism, and Fertility Disorders, and Pregnancy

Hypothyroidism is accompanied by an increased risk of fertility disorders and complications during pregnancy. [1, 2, 9] These are a consequence of hormonal changes in the form of a decrease in metabolic clearance of androstenedione and estrone and changes in SHBG levels leading to a reduction in testosterone and estradiol, with an increase in the free fraction of these hormones. The stimulatory effect of LH on TRH secretion and an increase in prolactin levels have also been shown in hypothyroidism. Elevated prolactin levels lead to ovulation disorders and corpus luteum insufficiency with low progesterone secretion in the luteal phase of the cycle. [1, 2, 3] Menstrual cycle disorders occur in 25-60% of women with hypothyroidism in relation to 10% of the healthy population. The most common is oligomenorrhea. There is no clear opinion on subclinical hypothyroidism vs fertility disorders, which is partly related to the different TSH cutoff values and the lack of prospective studies. A retrospective study in Denmark involving 11,254 women showed an increase in fertility disorders in subclinical hypothyroidism diagnosed at TSH levels below 3. 7 mIU/ml. [10] Analyzing the results of many papers, it is suggested that a TSH value below 4. 0 mIU/l may be a risk factor for fertility disorders. [1, 2] A significant factor that disrupts fertility is autoimmune thyroid disorders, which occur in about 10% of women. Many studies have shown that the presence of antibodies in euthyroid women was associated with fertility disorders.[1, 2] Elevated levels of anti-TPO antibodies are considered the most sensitive test for evaluating autoimmune disorders. It should be mentioned the presence of autoimmune thyroid disorders is a risk factor for miscarriages and premature births as well as in pregnancies achieved by ART. [1, 2] Overt hypothyroidism in pregnancy is diagnosed with TSH values above 2. 5 mIU/ml and decreased FT4 or TSH above 10. 0 mIU/ml regardless of FT4 values. In contrast, subclinical hypothyroidism is diagnosed with TSH values of 2. 5 – 10. 0 mIU/ml and normal FT4 levels. For women planning pregnancy, it is suggested to obtain TSH values below 2. 5 mIU/ml, preferably around 1. 0 mIU/ml. To achieve a TSH level of 2. 0-2. 5 mIU/ml, it is advisable to administer l-thyroxine, mainly if an elevated titer of anti-TPO antibodies is found. Once pregnancy is achieved, the dose of l-thy-

roxine should be increased by 30-50%. The TSH level should be checked every four weeks. After delivery, return to the starting dose and check TSH every 4-6 weeks. The use of L-tyroxine is not a contraindication to breastfeeding. [1, 2] In summary, in a woman who is planning a pregnancy or is pregnant to assess thyroid function, TSH, FT4, and FT3 determination should be performed; if hypothyroidism is shown, anti-TPO antibody level should be determined, and in case of hyperthyroidism TRAb. In the case of hyperthyroidism and pregnancy, you can use drugs according to the recommendations discussed above; in the case of hypothyroidism, there is no contraindication to the use of medications during pregnancy.

REFERENCES

1. Mata MAS, Ferreira AC, Carvalho DP, et al. Thyroid hormone resistance: Multicentric case series study. *Best Pract Res Clin Endocrinol Metab.* 2020; 34(4): 101378.
2. Hubalewska-Dydejczyk A, Duntas LH, Acharya S, et al. Thyroid disorders in pregnancy: Guidelines of the Polish Society of Endocrinology. *Endokrynol Pol.* 2021; 72(5): 425-436.
3. Hubalewska-Dydejczyk A, Lewiński A, Stasiak M, et al. Thyroid disease and fertility disorders. *Endokrynol Pol.* 2022; 73(4): 645-655.
4. Millar LK, Wing DA, Leung AS, et al. Low birth weight and preeclampsia in pregnancies complicated by hyperthyroidism. *Obstet Gynecol.* 1994; 84: 946-949.
5. Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. *Endocr Rev.* 2010; 31(5): 702-755.
6. Poppe K, Glinoer D, Tournaye H, et al. Thyroid dysfunction and autoimmunity in infertile women. *Thyroid*. 2002; 12(11): 997-1001.
7. Krassas GE, Pontikides N, Kaltsas T, et al. Menstrual disturbances in thyrotoxicosis. *Clin Endocrinol (Oxf).* 1994; 40(5): 641-644.
8. Sturgis SH, Woolridge RL, Rytand DA. The menstrual pattern in thyroid disease. *J Clin Endocrinol Metab.* 1952; 12(7): 846-855.
9. Van den Boogaard E, Vissenberg R, Land JA, et al. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: A systematic review. *Hum Reprod Update.* 2011; 17(5): 605-619.
10. Feldthusen AD, Laurberg P, Faber J. Impaired fertility associated with subclinical hypothyroidism and thyroid autoimmunity: The Danish General Suburban Population Study. *J Pregnancy.* 2015; 2015: 132718.

REPRODUCTIVE POTENTIAL AND PLOIDY COMPARISON OF 5, 6, AND 7-DAY BLASTOCYSTS: AN ANALYSIS OF IMPLANTATION, CLINICAL PREGNANCY AND LIVE BIRTH RATES

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SUMMARY

Extended culture to the blastocyst stage has become a widely adopted practice in assisted reproductive technologies (ARTs), offering significant improvements in embryo selection and supporting single embryo transfer. Conventionally, the selection of viable blastocysts occurs on Days 5 and 6 of embryo culture, with embryos deemed unsuitable for transfer, biopsy, or cryopreservation after Day 6 being routinely discarded. However, a subset of embryos exhibits slower development, reaching the blastocyst stage on Day 7. Remarkably, Day 7 blastocysts possess the potential for viability, as they can attain top morphological grades, display euploidy, and ultimately lead to healthy live births. Unfortunately, the prevailing practice in most clinics involves terminating culture on Day 6, which may result in prematurely discarding viable Day 7 blastocysts. While Day 7 blastocysts constitute only 5% of the total viable blastocysts, those that meet the criteria for cryopreservation or biopsy hold significant clinical value. Culturing embryos for an additional day extends the window of opportunity, allowing for identifying more usable embryos per the in vitro fertilization (IVF) cycle. This approach is particularly advantageous for patients with limited numbers or lower-quality blastocysts, providing enhanced prospects for successful pregnancies.

Keywords: Day 7 blastocyst, slow-growing blastocyst, delayed embryo development, live birth rate, euploidy, aneuploidy, clinical pregnancy rate

INTRODUCTION

Embryo selection is a crucial factor in the success of assisted reproductive technology (ART) treatments. The development of advanced embryo culture media has revolutionized in vitro fertilization (IVF) by enabling the extended culture of embryos to the blastocyst stage, resulting in improved embryo selection and higher implantation rates.1, 2, 3, 4, 5, 6 Furthermore, advance-

ments in cryopreservation techniques and preimplantation genetic testing for aneuploidy (PGT-A) have enhanced the selection of euploid embryos for transfer, leading to improved IVF cycle outcomes.[7, 8, 9, 10, 11, 12, 13]

While traditionally, embryo culture has been halted on day 6, recent studies have shed light on the potential of day 7 blastocysts and their viability for transfer.[14, 15, 16, 17, 18, 19] Early studies suggested lower pregnancy and live birth rates for day 7 blastocyst transfer, leading to a reluctance to culture embryos beyond day 6.[20,21] However, more recent investigations have reported viable pregnancies and live births with day 7 blastocysts, providing an opportunity for patients who lack day 5 or 6 embryos to achieve pregnancy.[19]

Despite the growing interest in extended embryo culture, data on outcomes associated with culture beyond day 6 are limited. Questions arise regarding the impact of prolonged embryo culture on embryo quality, the window of blastulation, and the range of time during which normal embryo blastulation occurs.[22, 23, 24, 25, 26] Additionally, the balance between optimizing the number of usable blastocysts and the risk of discarding potentially viable embryos needs to be addressed.[27]

This review article explores the outcomes and developmental potential of day 7 blastocysts. Through a comprehensive analysis of studies that include biopsy or cryopreservation of day 7 blastocysts, several vital clinical questions will be addressed, including the incidence of usable day 7 blastocysts, rates of euploidy, implantation and pregnancy rates, potential mechanisms for delayed blastocyst development, strategies for clinical implementation, and recommendations for future studies.

RESULTS

Association Between Embryo Euploidy and Biopsy Time

„What is the reproductive potential of day 7 euploid embryos?“

Day 7 blastocysts have a significantly lower euploidy rate than day 5 and day 6 blastocysts, indicating a higher likelihood of aneuploidy. A comprehensive analysis of IVF cycles from a single academic center, which included 25,775 embryos undergoing trophectoderm biopsy and preimplantation genetic testing for aneuploidy (PGT-A), revealed that only 40.5% of day 7 blastocysts were euploid. In contrast, the euploidy rates were notably higher for day 5 blastocysts at 54.7% and day 6 blastocysts at 52.9%. After accounting for potential confounding factors such as maternal age, anti-Müllerian hormone levels, BMI, embryo quality, and the number of embryos biopsied, the significant association between aneuploidy and day 7 biopsied embryos compared to day 5 and day 6 was evident.

„Day 7 blastocyst euploidy supports routine implementation for cycles using preimplantation genetic testing.“

Despite the lower euploidy rate, day 7 blastocysts can still be used in cycles with preimplantation genetic testing, as they have shown potential to develop into viable euploid embryos, providing additional opportunities for successful in vitro development and live births. A study investigating single, euploid frozen embryo transfers (FET) found that day 7 blastocysts, though accounting

for only 6.6% of all biopsied blastocysts, exhibited a substantial implantation potential of 35.9%. This finding indicates that even with a lower euploidy rate, day 7 blastocysts can contribute to successful pregnancies when selected for transfer in the context of PGT-A.

„Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study."

The study considered the impact of biopsy timing on blastocyst development and aneuploidy rates. Among the 1730 biopsied blastocysts, there were differences in euploidy rates based on the biopsy day. Specifically, 67.7% of blastocysts biopsied on day 5 were euploid, while only 52.1% and 43.1% of blastocysts biopsied on day 6 and day 7, respectively, were euploid (Day 5 versus Day 6: odds ratio (OR) 0.7, $P < 0.001$; Day 5 versus Day 7: OR 0.56, $P < 0.001$; Day 6 versus Day 7: OR 0.81, $P = 0.036$).

The results emphasize that the biopsy timing may influence the likelihood of obtaining euploid embryos. Additionally, the study found that aneuploidy risk increased with maternal age, with an approximate 10% increase in aneuploidy probability per year of maternal age (odds ratio (OR) = 1.1, $P < 0.001$).

„Earlier days of blastocyst development predict embryonic euploidy across all ages: essential data for physician decision-making and counseling patients."

Earlier blastocyst development is associated with a higher rate of embryonic euploidy, and this finding holds truth for both autologous and donor embryos. An extensive analysis of 2,132 biopsied blastocysts from 388 IVF cycles demonstrated a clear trend: blastocysts biopsied on days 5, 6, and 7 had euploidy rates of 49.5%, 36.5%, and 32.9%, respectively. This trend indicates that embryos biopsied at earlier stages of development have a higher likelihood of being euploid. Additionally, maternal age and the number of blastocysts biopsied per patient were identified as independent factors associated with a more significant percentage of euploidy, reinforcing the importance of considering these variables during embryo selection.

„Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as Day 5 and Day 6 blastocysts."

The prevalence of euploidy decreased with increasing time to embryo blastulation, reinforcing the importance of considering blastocyst development timing during preimplantation genetic testing. The results showed that day 7 blastocysts had lower euploidy rates (43.1%) compared to day 5 (67.7%) and day 6 (52.1%) blastocysts. These findings suggest that embryos taking longer to reach the blastocyst stage are more likely to be aneuploid. The prevalence of euploidy decreased with increasing time to embryo blastulation, reinforcing the importance of considering blastocyst development timing during preimplantation genetic testing. Based on the research results regarding euploidy, aneuploidy, and PGT-A (preimplantation genetic testing for aneuploidy), it can be concluded that day 7 euploid embryos have a lower reproductive potential compared to day 5 and day 6 embryos. The rate of embryo euploidy is significantly lower in day 7 blastocysts, indicating that a higher proportion of embryos biopsied on day 7 are likely to be aneuploid. However, despite the lower euploidy rate, day 7 blastocysts can still support routine implementation for

cycles using preimplantation genetic testing, and the extended culturing to day 7 can lead to the development of viable euploid embryos that would have otherwise been discarded.

IMPLANTATION AND CLINICAL PREGNANCY RATE

After analyzing the results from multiple research studies, several important conclusions can be drawn regarding the clinical contribution and reproductive potential of poor-quality blastocysts (PQBs) and day 7 euploid embryos in assisted reproductive technologies:

„Looking past the appearance: a comprehensive description of the clinical contribution of poor-quality blastocysts to increase live birth rates during cycles with aneuploidy testing."

Poor-quality blastocysts (PQBs) have proven to contribute to an impressive 12.4% increase in cycles leading to at least one live birth (LB) during aneuploidy testing. Nevertheless, it is essential to note that PQBs display slower development rates compared to their non-PQB counterparts. On average, each cycle yielded 0.7 ± 0.9 PQBs for biopsy, of which 0.2 ± 0.4 were euploid. The prevalence of PQBs is influenced significantly by maternal age, with women over 42 years benefiting more from these embryos. Intriguingly, 18 women achieved their only live births thanks to PQBs. Furthermore, patients with limited or no sibling non-PQBs also experienced higher success rates in achieving successful pregnancies through PQBs.

„Cryopreserved embryo transfers suggest that endometrial receptivity may contribute to reduced success rates of later developing embryos."

Observations from research on cryopreserved embryo transfers suggest that endometrial receptivity might reduce success rates for later developing embryos. Clinical pregnancy rates (PRs) were comparable between blastocysts cryopreserved on day 5 and day 6 (32% vs. 28%). However, blastocysts cryopreserved on day 7 displayed a lower clinical PR (15%). Although this difference initially appeared significant after accounting for the number of embryos per transfer, statistical analysis showed that the discrepancy was not statistically significant (P = 0.15).

„What is the reproductive potential of day 7 euploid embryos?"

The analysis of the reproductive potential of day 7 euploid embryos revealed significant distinctions among cohorts in terms of implantation, clinical pregnancy, live birth, and clinical loss rates. Compared to day 5 and day 6 embryos, day 7 biopsied embryos exhibited decreased odds of implantation, clinical pregnancy, and live birth. The implantation rate for day 5 blastocysts – 65.4%, day 6 – 56,2%, day 7 – 30.1%. However, no significant association was observed with clinical loss or multiple pregnancy rates in patients utilizing day 7 embryos during treatment.

„Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as Day 5 and Day 6 blastocysts."

Despite this lower euploidy rate, day 7 euploid embryos displayed a sustained implantation rate (SIR) similar to day 5 and day 6 embryos. For euploid single embryo transfers (SET), the SIR for day 5 and day 6 embryos was approximately 68.9% and 66.8%, respectively. Although day 7 euploid SET showed a slightly lower SIR at 52.6%, this difference did not reach statistical signifi-

cance. The collective research findings emphasize the positive clinical contribution of poor-quality blastocysts in increasing live birth rates during cycles with aneuploidy testing, particularly benefiting women over 42 years old and those with limited sibling non-PQBs. However, it is crucial to acknowledge that PQBs display slower development and lower euploidy rates than nonPQBs. Day 7 euploid embryos, despite their lower euploidy rates, exhibit comparable sustained implantation rates as day 5 and day 6 embryos. Considering these factors is vital for assisted reproductive technology clinics to optimize success rates when selecting and transferring blastocysts for their patients.

LIVE BIRTH RATE

„What is the reproductive potential of day 7 euploid embryos?“

A sub-analysis of 3824 single, euploid frozen embryo transfer (FET) cycles was conducted, classifying them based on the day of blastocyst development: day 5 (n = 2321 cycles), day 6 (n = 1381 cycles), and day 7 (n = 116 cycles). Significant differences were observed among these groups in implantation, clinical pregnancy, live birth (LB), and clinical loss rates. The odds of implantation, clinical pregnancy, and LB were significantly lower in patients who utilized day 7-biopsied embryos during treatment. The live birth rates for day 5, day 6, and day 7 groups were 56.4%, 45,8%, and 21.5%, respectively.

„Worth the wait?

Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates to Day 5 and Day 6 blastocysts“ Day 7 blastocysts have lower live birth rates than Day 5 and Day 6 blastocysts. Although they offer viable euploid embryos that would have otherwise been discarded, the extended culture to Day 7 is associated with increased miscarriage rates. The live birth rates for Day 7 blastocysts were significantly lower at 43.8% compared to 67.4% and 77.2% for Day 5 and Day 6 blastocysts, respectively.

„Euploid day 7 blastocysts of infertility patients with only slow embryo development have reduced implantation potential.“

In this study, a total of 2966 women underwent single euploid FET using embryos that reached blastulation on either day 5 (n = 1880), day 6 (n = 986), or day 7 (n = 100). The results showed that day 7 embryos had significantly lower implantation and live birth rates compared to both day 5 and day 6 embryos ($P < 0.001$). The live birth rates for the day 5, day 6, and day 7 groups were 68.5%, 55.2%, and 36.0%, respectively. Furthermore, the day 7 group was older than the day 5 group ($P < 0.001$). Even after comparing age-matched cohorts, the day 7 group still exhibited lower implantation and live birth rates ($P < 0.0001$ and $P < 0.001$, respectively). Interestingly, the study found that the live birth rates were not influenced by embryo grade.

CONCLUSION

Maternal age, culture medium, blastocyst expansion time, and biopsy timing influence the prevalence of euploidy, implantation potential, and live birth rates. These findings highlight the im-

portance of considering extended culture and blastocyst development on Day 7 in ART practice. Day 7 blastocysts can be of high quality and genetically normal. They can potentially result in healthy live births following frozen embryo transfer. However, further research is needed to determine the success rates and live birth rates of Day 7 blastocysts, considering factors such as blastocyst grade, ploidy result, and patient age. Further research is needed to accurately define the Day 7 blastocysts' success rates and incorporate them into clinical practice. It is recommended to consider extended culture to Day 7, especially for embryos with early or borderline grades on Day 6, as it may increase the live birth rate per cycle. Additionally, the management of Day 7 blastocysts should involve setting patient expectations and considering individual patient characteristics.

AUTHORS' ROLES

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Sunde A, Brison D, Dumoulin J. Quality control in IVF: Towards a more comprehensive evidence-based approach. *Hum Reprod.* 2016; 31(3): 477-482.
2. Taylor TH, Gitlin SA, Patrick JL. The current state of evidence on elective embryo cryopreservation: a systematic review with quality assessment of the literature. *Fertil Steril.* 2014; 102(4): 988-996.
3. Minasi MG, Colasante A, Riccio T, et al. Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study. *Hum Reprod.* 2016; 31(10): 2245-2254.
4. Gardner DK, Lane M, Schoolcraft WB. Physiology and culture of the human blastocyst. *J Reprod Immunol.* 1998; 39(1-2):149-164.
5. Lane M, Gardner DK. Understanding cellular disruptions during early embryo development that perturb viability and fetal development. *Reprod Fertil Dev.* 2007; 19(6): 681-688.
6. Smith GD, Takayama S, Swain JE. Rethinking in vitro embryo culture: new developments in culture platforms and potential to improve assisted reproductive technologies. *Biol Reprod.* 2012; 86(3): 62.
7. Scott RT, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril.* 2012; 100(3): 697-703.
8. Kang HJ, Melnick AP, Stewart JD, Xu K, Rosenwaks Z, Palermo GD. Preimplantation genetic screening: who benefits? *Fertil Steril.* 2016; 106(3): 597-602.

9. Treff NR, Zimmerman RS. New insights into embryo selection for in vitro fertilization. *Fertil Steril.* 2017; 107(3): 621-627.
10. Lawrenz B, Fatemi HM. Effect of embryo cryopreservation techniques on ART outcomes. *Nat Rev Endocrinol.* 2017; 13(7): 429-438.
11. Al-Azemi M, Killick SR, Duffy S, et al. A multi-centre analysis of the relative effectiveness of cryopreserved versus fresh blastocyst transfer. *Reprod Biomed Online.* 2012; 25(6): 566-572.
12. Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum Reprod.* 2016; 31(10): 2243-2254.
13. Wang L, Cram DS, Leigh D. Advances in preimplantation genetic testing for monogenic disease and aneuploidy. *Best Pract Res Clin Obstet Gynaecol.* 2017; 44: 62-74.
14. Hiraoka K, Hiraoka K, Horiuchi T, Shioya Y. Blastocysts become arrested and/or degenerate in vitro on day 6. *J Assist Reprod Genet.* 2008; 25(2-3): 95-99.
15. Wirleitner B, Vanderzwalmen P, Stecher A, et al. Selection of embryos on day 5 for transfer is associated with increased implantation rates. *Reprod Biomed Online.* 2016; 32(4): 422-428.
16. Tiegs AW, Hartshorne GM, Goulding AM. Blastocyst development rate influences implantation and live birth rates of similarly graded euploid blastocysts. *Fertil Steril.* 2018; 109(6): 1028-1034.
17. Hammond ER, McGillivray BC, Wicker SM, Peek JC. The influence of blastocyst transfer on singleton fetal growth trajectory and placental morphometry. *Fertil Steril.* 2018; 110(3): 483-492.
18. Du H, Sun X, Gao X. Pregnancy and neonatal outcomes of blastocyst transfer on day 7. *J Assist Reprod Genet.* 2018; 35(6): 1043-1049.
19. Whitney JB, Foster JS, McGovern PG. Outcomes of day 7 blastocysts at a university-based ART program. *J Assist Reprod Genet.* 2019; 36(4): 739-744.
20. Shoukir A, Campana A, Goulard M. Day 7 blastocyst transfer: pregnancy and live birth rates. *Fertil Steril.* 1998;69(4):712-715. doi:10.1016/S0015-0282(98)00044-5.
21. Utsunomiya T, Nakagawa K, Fuchinoue K. Day 7 blastocyst culture and transfer: a study of pregnancy and live birth rates. *Hum Reprod.* 2004;19(2):285-291. doi:10.1093/humrep/deh026.
22. Navot D, Bergh PA, Williams MA. Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. *Lancet*. 1991; 337(8754): 1375-1377.
23. Prapas Y, Prapas N, Diedrich K. Blastocyst culture and transfer: a review of clinical outcomes. *Hum Reprod.* 1998;13(5):1332-1335. doi:10.1093/humrep/13.5.1332.
24. Bosch E, Valencia I, Mendoza C, et al. Day 5 vs. day 6 blastocyst transfer: a randomized controlled trial. *Fertil Steril.* 2010;93(2):549-553. doi:10.1016/j.fertnstert.2008.12.078.
25. Marek D, Hawkes M, Johnson N. Comparative study of blastocyst culture to day 6 versus day 5: outcomes and implications. *J Assist Reprod Genet.* 2001;18(7):307-313. doi:10.1023/A:1012607521474.
26. Kovalevsky G, Kagan K, Azziz R. Outcomes of day 7 blastocyst transfer and implications for clinical practice. *Reprod Biomed Online.* 2013;27(3):301-308. doi:10.1016/j.rbmo.2013.06.001.

27. Sunkara SK, Rittenberg V, Li J, et al. The effect of embryo culture to the blastocyst stage on live birth rates: a meta-analysis. *Hum Reprod.* 2010;25(2):423-431. doi:10.1093/humrep/dep389.
28. Insogna IG, Lanes A, Ginsburg ES, Racowsky C. Quality of embryos on day 7 after medium refreshment on day 6: a prospective trial. *Hum Reprod.* 2021; 36(5): 1253-1259.
29. Hernandez-Nieto C, Lee JA, Slifkin R, et al. What is the reproductive potential of day 7 euploid embryos? *Hum Reprod.* 2019; 34(9): 1697-1706.
30. Cimadomo D, Soscia D, Casciani V, et al. How slow is too slow? A comprehensive portrait of Day 7 blastocysts and their clinical value standardized through artificial intelligence. *Hum Reprod.* 2022; 37(6): 1134-1147.
31. Fraire-Zamora JJ, Serdarogullari M, Sharma K, et al. Better late than never: the clinical value of Day 7 blastocysts. *Hum Reprod.* 2023; 38(3): 520-524.
32. Tiegs AW, Sun L, Patounakis G, Scott RT Jr. Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as Day 5 and Day 6 blastocysts. *Hum Reprod.* 2019; 34(9): 1632-1639.
33. Hammond ER, Cree LM, Morbeck DE. Should extended blastocyst culture include Day 7? *Hum Reprod*. 2018; 33(6): 991-997.
34. Richter KS, Shipley SK, McVearry I, Tucker MJ, Widra EA. Cryopreserved embryo transfers suggest that endometrial receptivity may contribute to reduced success rates of later developing embryos. *Fertil Steril.* 2006; 86(4): 862-866.
35. Lane SL, Reed L, Schoolcraft WB, Katz-Jaffe MG. Euploid day 7 blastocysts of infertility patients with only slow embryo development have reduced implantation potential. *Reprod Biomed Online.* 2022; 44(5).
36. Hiraoka K, Hiraoka K, Miyazaki M, et al. Perinatal outcomes following transfer of human blastocysts vitrified at days 5, 6 and 7. *J Exp Clin Assist Reprod.* 2009; 6(4).
37. Capalbo A, Rienzi L, Cimadomo D, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study involving 956 screened blastocysts in two centers. *Hum Reprod.* 2014; 29(6): 1173-1181.
38. Cimadomo D, Soscia D, Vaiarelli A, et al. Looking past the appearance: a comprehensive description of the clinical contribution of poor-quality blastocysts to increase live birth rates during cycles with aneuploidy testing. *Hum Reprod.* 2019; 34(7): 1206-1214.

ELECTROMYOGRAPHIC EVALUATION OF THE PELVIC MUSCLES ACTIVITY AFTER HIGH-INTENSITY FOCUSED ELECTROMAGNETIC PROCEDURE AND ELECTRICAL STIMULATION IN WOMEN WITH PELVIC FLOOR DYSFUNCTION

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SUMMARY

Electromyography (EMG) effectively measures pelvic floor muscle (PFM) activity, revealing differences in muscle function in women with pelvic floor dysfunction (PFD). This study compares the efficacy of high-intensity focused electromagnetic (HIFEM) therapy and traditional electrical stimulation in treating PFD. Surface EMG and subjective assessments (Pelvic Floor Impact Questionnaire, PFIQ) were used to evaluate PFM strength, endurance, and relaxation changes. Results showed that HIFEM significantly improved muscle activation, relaxation, and endurance compared to electrical stimulation. The PFIQ scores also indicated a more significant improvement with HIFEM, suggesting it is more effective than electrical stimulation for restoring PFM strength and alleviating PFD symptoms. These findings highlight HIFEM's superior efficacy in PFM rehabilitation, offering a promising alternative for postpartum women and those with urinary incontinence or sexual dysfunction.

Keywords: Electrical stimulation, electromyographye HIFEM procedure, pelvic floor dysfunction; pelvic floor muscles

INTRODUCTION

Electromyography (EMG) is a method frequently used to examine the electrical activity of muscle tissue. Although this technology is relatively new, it is assumed to be reliable and objective while causing minimal or no discomfort to patients. Essentially, EMG uses the surface or intramuscular electrodes to record the intensity of signals propagating in the muscle fibers during the contraction because muscle tissue conducts electrical potentials similar to the nerves. The results of the measurements are expressed as a function of voltage over time. Except for single-fiber EMG, one

measured value represents a sum of all signals originating from the muscle tissue of a specific body area.[2-4]

Besides ultrasound,[5,6] magnetic resonance,[7] manometers,[8] dynamometers,[9] or simple palpation combined with observation,[10] surface EMG (sEMG) is one of the possible objective methods for monitoring resting level, strength, and endurance of the pelvic floor muscles (PFMs). The pelvic floor consists of 3 main compartments – anterior (bladder and urethra), middle (vagina and uterus), and posterior (rectum). Furthermore, there are morphologically complex multilayers of anatomical structures such as the pelvic diaphragm (composed of the levator ani and coccygeus muscles), urogenital diaphragm (consisting of connective tissue, perineum, bulbospongiosus, and ischiocavernosus muscles), and urethral/anal sphincters. These tissues are arranged in the pelvic area and have multiple attachments to the surrounding structures.[1] Under normal circumstances, the PFM prevents multiple disorders such as incontinence (urinary/fecal), sexual dysfunction, or pelvic organ prolapse accompanied by pain and discomfort. However, the atrophy and relaxation of PFMs may promote the manifestation of these health issues, collectively referred to as pelvic floor dysfunction (PFD),[10-12] occurring naturally with ageing or as a consequence of childbirth. Recording of sEMG in women who showed specific symptoms of PFD was reported previously by multiple authors. It has been found that EMG is a suitable method for the investigation of PFM functioning among healthy subjects and women with signs of urinary incontinence or PFM weakness.[13-21] Despite the various protocols and electrode configurations used, there is a clear relationship between the characteristics of the EMG signal and PFD. In comparison with the healthy and asymptomatic subjects, postmenopausal and even premenopausal women affected by some form of PFM impairment show distinctively lower EMG values. The intensity of maximum voluntary contraction (MVC) is reduced because the PFMs are weakened, and the endurance of contraction and muscle activity during rest are also affected.[13,14,18-20] Aside from sEMG, various subjective questionnaires (Pelvic Floor Disability Index, Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire, Pelvic Floor Impact Questionnaire, International Consultation on Incontinence Questionnaire Vaginal Symptoms or Pelvic Floor Bother Questionnaire) were also used to document strengthening and reeducation of the PFM which helped patients to improve their symptoms.[22,23] Besides regular exercise,[24] the function of the weakened PFM can be enhanced by noninvasive PFM stimulation. With well-established electrical stimulation,[25,26] high-intensity focused electromagnetic (HIFEM) technology has been more frequently used in recent years.[27-29] Both technologies deliver electric currents into the pelvic floor to depolarize membranes of motoneurons to elicit action potential and achieve brain-independent muscle contractions when the action potential of sufficient strength reaches the neuromuscular junction.[30] However, despite the direct flow of electric charge through the electrode-tissue surface, the HIFEM induces electrical currents selectively in the PFM by the mechanism of electromagnetic induction.[31] As the magnetic field passes any medium without energy attenuation, the induced contractions may be achieved at greater depths and intensities[32] to provide better outcomes. Based on the previous rationale, this study aims to investigate and compare treatment outcomes of the HIFEM procedure and electrical stimulation in women suffering from PFD. The expected changes in PFM activity would be examined by subjective (Questionnaire) and objective (sEMG) methods. The measured values will be compared with asymptomatic subjects.

MATERIALS AND METHODS

Patient's Recruitment Criteria

The inclusion criteria were specified as follows: women of age 18-45 years, who had a vaginal delivery, and who already stopped lactation. There were three patient groups. The symptomatic patients who reported PFD symptoms related to weakened PFM as lower urinary tract or bowel symptoms (incontinence) and/or sexual dysfunction (dyspareunia, vaginal laxity, decreased sensitivity during intimacy, inability to achieve orgasm – anorgasmia) were randomly (2:1) divided into the G1 group treated by HIFEM and G2 group which received electrical stimulation. The third group, G3, consisted of healthy postpartum patients to obtain sEMG values of the normal population. Exclusion criteria were the presence of any metal implants or devices, which include metal components, pregnancy, malignant tumor, history of surgical procedure in the pelvic region, presence of pelvic organ prolapse of stage II-IV as per the Pelvic Organ Prolapse Quantification classification, and all general contraindications for physiotherapy. Patients were asked to perform pregnancy tests before the first treatment and then retest regularly.

Considerations

This study was approved by the local ethics committee of Hospital Lapino (MD medical group). It complied with ethical principles stated in the Declaration of Helsinki, Convention on Human Rights and Biomedicine, and International Ethical Guidelines for Health-related Research Involving Humans, and it completely excludes impairment of patients' interests and damage to health. All subjects were informed about the study's potential risks and possible benefits, and all participants provided written informed consent.

Treatment Protocol

Both intervention groups received ten treatments in total addressing the stimulation of PFM. The G1 group was treated using a BTL EMSELLA (BTL Industries Inc, Boston, MA) device, which uses HIFEM technology for noninvasive PFM stimulation and reeducation based on the principle of electromagnetic induction. The device consists of a generator connected to the chair where the stimulation coil is located. The coil emits a focused magnetic field of intensities up to 2.5 Tesla, responsible for the induction of muscle contraction up to depths of 10 cm. Each therapy with the BTL EMSELLA device lasted 28 minutes and was administered under a skilled physician's supervision at the Lapino Hospital. Patients were seated in a chair, and the intensity of the stimulus was modulated on the scale of 0-100% (0-2.5 Tesla) by their feedback up to the maximum tolerable threshold when patients felt a strong muscle contraction but without pain or discomfort. All patients have achieved 100% intensity during the first or second procedure. Treatments with HIFEM were addressed 2-3 times per week for four weeks. The sessions were planned to suffice this interval per the patient/device availability. Two consecutive treatments were spaced at least 48 hours apart to prevent muscle fatigue. The G2 group performed home-based and self-administered procedures with a BioBravo (MTR Vertriebs, GMBH, Germany) electrical stimulation device:

1. The patients were comprehensively trained to safely and effectively use a BioBravo stimulator.

2. They were instructed to finish treatments at home by repeating therapy every other day. The stimulation protocol was identical for both groups because the settings of the BioBravo device were adjusted to reflect those used by the BTL EMSELLA device.
3. Group G3 did not receive any treatment.

sEMG Measurements

The study's primary outcome was to perform sEMG measurements to determine the activation of the PFM in symptomatic and asymptomatic patients and to document the hypothesized changes caused by muscle strengthening. At first, by using a Myomed 632 myofeedback device (Enraf-Nonius B.V., Netherlands), the patients were instructed on how to correctly perform contractions of the PFM without (voluntary) involving the muscles of the anterior abdominal wall and gluteal or hip region. When performing contractions, patients were lying in the supine position. During the examination, they were requested to repeat three specified PFM activations, which consisted of the following: five short (quick flick) contractions at maximum intensity with an interval of 10 seconds, followed by sustained contractions and relaxation (both 10 seconds long, five repetitions) and finally the sustained contraction held as long as possible to determine PFM endurance.[33] The sEMG recordings were performed by the Myomed 632 device at the baseline (all groups) and after the patient's last treatment (only G1 and G2). To isolate the signal originating in the PFM, two types of superficial electrodes were used: the first was applied on the anterior abdominal area (served as reference), and the second (vaginal) electrode was mounted on the intravaginal probe. The neutral gel was always applied on the sensor introduced into the vagina. An experienced physiotherapist confirmed the correct placement of the intravaginal probe and PFM contractions. Concurrent registration of muscular electric potential by using the vaginal and skin electrodes allowed differentiating PFM contractions. During the sEMG examination, myofeedback (in the form of a graph) was displayed on the device's monitor and the external monitor unit connected to the device to enlarge the graphic output. The sEMG measurements were performed automatically by the Myomed device, following the pattern of PFM activations described above. These parameters were acquired for each patient during each visit: MVC, mean MVC, mean activity at rest/resting level (all in mV), and endurance of contraction (in seconds).

Standardized Questionnaire

The secondary outcome was to assess subjective changes in the perception of PFD by the PFIQ-7. This standardized Questionnaire was used to determine the impact of PFD on the patient's quality of life, as it was shown to be psychometrically valid and reliable in previous research.[34] Patients from groups G1 and G2 were given the PFIQ-7 at baseline and after the last treatment. Based on their answers, the PFIQ mean scores (on a scale from 0 = no distress to 300 = maximal distress) were calculated and compared against baseline and between both groups.

Safety

The safety of treatments, sEMG measurements, and possible adverse events (AEs) were monitored. Patients were also asked to report any signs of discomfort or pain during the therapies or caused by the positioning of the intravaginal electrode.

Statistical Analysis

All variables were checked for normality using the Kolmogorov-Smirnov test. Descriptive statistics were estimated by the sample mean with a 95% confidence interval. The differences between groups were tested using an analysis of variance test followed by Least Significant Difference post hoc tests. Levene's variance homogeneity test was run before variance analysis to verify the equal variances in groups. A student's t-test tested paired variables. All statistical tests were 2-tailed. A whole statistical analysis was conducted with Statistica v.6 (StatSoft Inc, Tulsa, OK), and the significance level was set as default to 0.05 (5%). Initially, the minimum sample size was verified by using Statistica software. At least 19 subjects must have been included in the three tested groups to achieve a power of 80% with a = 5%.

RESULTS

Patient Group Characteristics

In total, 95 patients were recruited between 2018 and early 2019 following the specified criteria and the current state of patients in the clinic: G1 (n=50), G2 (n=25), and G3 (n=20). See Table 1 for detailed characteristics of patient groups. All recruited patients from the G1 and G2 groups finished a prescribed number of treatment sessions. Eight patients with zero PFIQ-7 score at the baseline (G1=5, G2=3) were excluded from the questionnaire evaluation. No AEs were observed regarding the delivered treatments or sEMG measurements. Subjects seldom reported only mild discomfort when recording sEMG using an intravaginal electrode.

Table 1. ***Characteristics of patient groups at the time of recruitment (mean followed by 95% confidence interval)***

Group	Age (years)	BMI (kg/m2)	Vaginal deliveries	PFD symptoms (% of patients)
G1 (n = 50)	31.12 (1.52)	23.27 (0.76)	1.76 (0.22)	Urinary incontinence (74%); decreased sexual desire (36%); decreased sensitivity during intimacy (70%); dyspareunia (26%); hypo/anorgasmia (52%)
G2 (n = 25)	31.96 (3.20)	24.32 (3.70)	1.56 (0.27)	Urinary incontinence (72%); decreased sexual desire (44%); decreased sensitivity during intimacy (44%); dyspareunia (24%); hypo/anorgasmia (40%)
G3 (n = 20)	27.20 (2.02)	22.40 (1.27)	1.25 (0.21)	-

BMI = body mass index; PFD = pelvic floor dysfunction.

Quantification of the EMG Signal

The results of sEMG measurements are summarized in Table 2. In general, there are significant differences between the symptomatic groups in comparison with healthy patients. On the other hand, the changes in the measured values after the HIFEM or electrical stimulation were highly

statistically significant (P < .001) in comparison with the baseline, showing that stimulation of the PFM modifies the muscle (electrical) activity.

At baseline, the measured peak intensity of the MVC signal was significantly higher in healthy patients by approximately 22 mV on average than in the G1 or G2 group. At the same time, there was no change between the intervention groups. At the end of the study, the G1 group showed significantly higher EMG values than the G2 group (P < .001), reaching an average change of 10.58 mV (57.29%) and 1.44 mV (7.34%), respectively. Although the HIFEM treatment considerably increased the PFM activity, the G1 group still showed lower values than the control. Similar findings were observed in the case of average MVC. As expected, the average MVC magnitudes are lower in each group. A more profound increment was also observed in the G1 group (6.65 mV, 58.69%) compared with a modest increase in the G2 group (0.91 mV, 6.81%). There were also significant differences between the G1 and G2 groups after treatments (P < .05). Despite the observed improvement, asymptomatic subjects still showed greater EMG values.

Interestingly, the examination of muscle activity at rest revealed divergent tendencies. Initially, only the G1 group showed significantly different (higher) values from the control (P < .05), while after the last therapy, the G1 average resting level decreased at the level of G3 (2.08 mV and 1.90 mV, respectively). Conversely, the average resting level of the G2 group had risen from 2.42 mV to 3.94 mV. In conclusion, the G2 subjects manifested significantly higher EMG values than the control and G1 groups at the end of the study (P < .001). Regarding endurance, significant differences were observed between the symptomatic and the control groups at the baseline and after the treatments (see Table 2). The measurement of the G3 group showed that healthy patients could hold the contraction of the PFM on average for 62.25 seconds.

Furthermore, we observed a significant increase in the endurance of PFM contraction by 48.24% in the G1 group because the patients could hold a contraction by 13.44 s longer after their treatments, reaching 41.30 s in total. The G2 group improved by 36.26%, and PFM contraction was prolonged on average by 6.60 s.

Table 2. ***Results of the sEMG measurements at the baseline and after the last therapy for both treated groups (G1 and G2) and control subjects (G3) are presented as mean followed by a 95% confidence interval in brackets***

Measurement	Group	Baseline	After
Peak MVC (mV)	G1 (n=50)	19.49 (2.31)	30.06*** (3.75)
	G2 (n=25)	19.56 (2.93)	21.00 (2.82)
Average MVC (mV)	G1 (n=50)	11.33 (1.54)	17.99†,* (2.50)
	G2 (n=25)	13.39 (2.46)	14.30 (2.42)
Resting level (mV)	G1 (n=50)	3.83†,* (0.82)	2.08 (0.38)
	G2 (n=25)	2.42 (0.45)	3.94†,*** (0.60)
	G3 (n=20)	1.90 (0.63)	-
Endurance (s)	G1 (n=50)	27.86†,** (4.17)	41.30†,*** (5.21)

Measurement	Group	Baseline	After
	G2 (n=25)	24.80 (3.12)	32.69 (1.88)
	G3 (n=20)	41.96 (2.51)	62.25 (3.68)

*P < .05, **P < .01, ***P < .001.

Pelvic Floor Impact Questionnaire Short Form 7

The patient's subjective evaluation is summarized in Table 3 and Figure 1. The minimal variation in the baseline score of both symptomatic groups was insignificant. Nonetheless, after the last treatment, there was a significant difference in the PFIQ score between the G1 and G2 groups (P < .01). Although both treatment modalities resulted in highly substantial subjective improvement, the patients treated with HIFEM experienced more remarkable outcomes. In addition, 16 patients (35.56%) from the G1 group reached a score of zero after the HIFEM treatments (meaning 100% improvement against the baseline). Contrary to this, only three patients (12.00%) from the G2 group who underwent electrical stimulation reported zero scores at their last visit. The shift in PFIQ scores is visualized in Figure 1. As can be seen, the relative frequency of scores was remarkably changed in the G1 group, while almost 90% of patients fell into the low score categories (0-10 or 10-20) after the treatments. In addition, scores of more than 50 were eliminated from patient's responses. The G2 group showed minimal changes in patients' PFIQ scores distribution, corresponding to a moderate average improvement of 5.15 points (see Table 3).

Table 3. ***Results of the Pelvic Floor Impact Questionnaire Short Form 7 (PFIQ-7) for both treated groups (G1 and G2) at baseline and after the last therapy session presented as mean followed by a 95% confidence interval in brackets***

Group	Baseline	After
G1 (n=45)	37.16 (4.68)	15.95 (2.55)
G2 (n=22)	32.28 (5.92)	21.96 (3.37)

DISCUSSION

Our examination of PFM electrogenesis in patients who showed signs of PFD revealed a significant reduction of the generated EMG signal compared with the asymptomatic patients at baseline (MVC, mean MVC, and endurance). The results of intervention groups G1 and G2 denote that noninvasive PFM strengthening can positively influence PFM activity. As seen in Table 2, the sEMG measurements obtained after therapies with the BTL EMSELLA device or electrical stimulation showed increased values of maximum possible voluntary contraction and endurance. It suggested that at the end of the study, patients could have more robust and more complex PFM contractions, resulting in a reduction of PFD symptoms (whether incontinence or sexually based), also demonstrated by a significant decrease in the PFIQ-7 score.

In contrast to sEMG measurements, which demonstrated considerable PFM weakening in the G1 and G2 groups at baseline, the PFIQ resulted in relatively low scores in both groups. We attribute this to perhaps a less specific grading system of the PFIQ when evaluating patients who showed various PFD-related symptoms of different severity. In future studies, it might be beneficial to focus on evaluating a particular patient's symptoms by using condition-specific questions assessed by a visual analog scale or a 5 to 7-point Likert scale, for instance, to enhance grading possibilities.

A magnetic and electrical stimulation comparison showed a significant improvement in EMG values, observed in the G1 group, which was treated by HIFEM technology. Compared with electrical stimulation, the BTL EMSELLA device was shown to be substantially more effective in restoring muscle strength as the MVC, mean MVC and endurance parameters uniformly increased from 48 to 59% after HIFEM treatments. On the contrary, electrical stimulation induced only mild changes in MVC (7.34%) or mean MVC (6.81%) while reaching mild to moderate improvement (36.26%) in endurance. The sEMG measurements coincide with the results of the PFIQ. The patient's subjective evaluation showed more pronounced improvement in the G1 group (57.16%) than in the G2 group (32.18%), corresponding to the improvement rate in EMG values. The HIFEM procedure also substantially reduced high PFIQ scores after the last therapy session (see Figure 1).

PFM Electrical Activity and sEMG Measurements

Given the specific patient group and scarce evidence in the literature, control group G3 was established to obtain normative EMG values that were valid for the studied sample. In general, the results presented here coincide with the previously published findings. It has been documented by numerous authors[13-15,17,18,20] that women who are suffering from PFD show lower MVC and endurance values because of the impairment of the PFM. Properly stimulating the PFM allows patients to produce more significant voluntary contractions for longer durations. In addition, the PFD influences muscle activity at rest as the PFMs are less electrically active.

However, the PFM resting level evaluation revealed significant differences between both modalities in our study. Although the G1 group, after treatments, reached similar EMG values as the healthy population, patients from group G2 showed altered muscle activation with relatively high electromyogenesis at rest (3.94 mV on average, see Table 2). This indicates that G2 patients cannot correctly relax their PFM after treatments because they cannot isolate and control the appropriate muscle activation patterns, which was then reflected by the lower MVC amplitudes. The correct activation pattern during PFM contraction is associated with increased activation of the PFM and lower transverse abdominal wall with markedly less activation of the upper abdominal and chest wall. The inappropriate activation refers to increased abdominal and chest wall activation while PFM activation decreases,[16] resulting in lessened strength (MVC amplitude) of contraction.

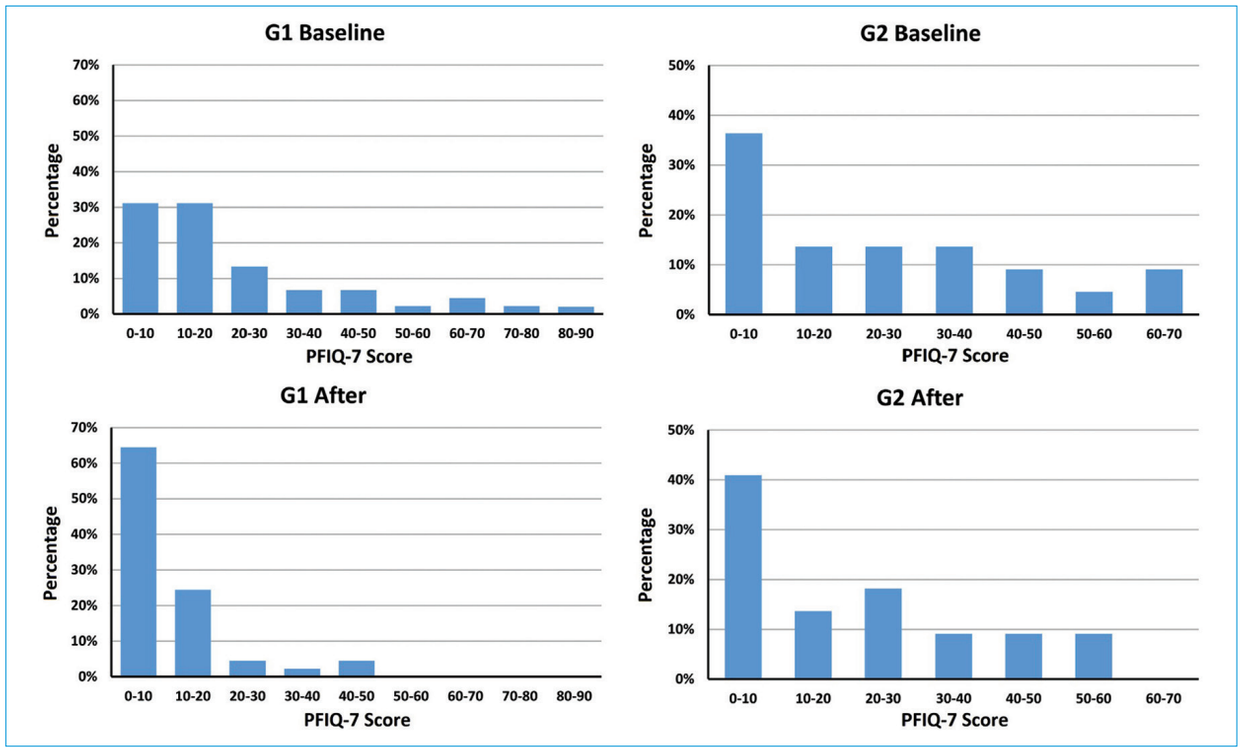
Showing high test-retest reliability,[13,14] the sEMG measurement is a valuable tool for detecting PFM activity. To record PFM electrical activity, we used an intravaginal electrode with a large surface to obtain EMG signals of sufficient amplitude with high sensitivity.[2,3] Fortunately, the PFM encompasses only a partial amount of subcutaneous tissue, possibly further attenuating the amplitude of EMG.[35] To prevent any systematic error during measurements, the skilled physiotherapist supervised the insertion and the position of the measuring electrode. Data normalization was

unnecessary as we assessed the same muscle group during one measurement session without removing the active electrode.[3] The selectivity of measured values was accomplished by the reference electrode placed on the abdomen. The signal obtained by the abdominal electrode was subtracted from the recording site to eliminate standard components and received EMG values, thus representing the summation activity of the whole PFM. To achieve an even greater degree of selectivity, the specific design of the vaginal electrode is required. For instance, Voorham-van et al.[14] have been able to successfully measure and compare the activity of selected pelvic muscles (pubococcygeus, puborectalis, bulbospongiosus, and ischiocavernosus) by using an experimental intravaginal probe with a matrix of 24 electrodes.

Study Limitations

Still, an sEMG measurement faces various challenges. The nature of the recorded electrical signal (amplitude, frequency, or noise) is influenced by several factors, such as the composition of measured muscle and the structure and position or placement of electrodes.[35] The core and skin temperature[36] or different humidity of measured environments may also influence the signal parameters. Because of the moisture and temperature within the vaginal lumen, it is challenging to ensure identical conditions at each visit during the intravaginal measurements. The moisture between the electrode and tissue may lead to decreased EMG amplitude. Furthermore, the electrode positioning is crucial for the reliability of sEMG measurement. Therefore, the operator must consistently insert the intravaginal probe into the measured muscles, as the electrode orientation affects the signal's power.[37] In addition, the intravaginal probes should be designed in such a way

Figure 1. ***The comparison of PFIQ-7 scores per group and appointment. The relative frequencies of scores reported by the patients of group 1 (G1) and group 2 (G2) are plotted in the graphs. There is a substantial shift toward the lower PFIQ-7 scores in the G1 group after the treatments.***



as to minimize any impact on the PFM by its insertion to avoid cross-talk and motion artifacts.[14] Indisputably, the appropriate planning of treatments is essential to achieve desired results. Unlike electrical stimulation, HIFEM is a relatively new technology that is still being investigated to some extent. In our study, the HIFEM treatments were administered at least 48 hours apart (2-3 per week) to maximize treatment outcomes and avoid muscle fatigue caused by overtreatment of the PFM, as the therapy with maximum settings produces intense muscle contractions. Presumably, the results would differ because of changes in the treatment frequency; however, this should be verified by future studies.

CONCLUSION

Electromyographic measurement of PFM activity proved to be a valid method for examination of patients with PFD (suffering from urinary incontinence and/or accompanied by sexual dysfunction) treated with HIFEM and electrical stimulation. Surface EMG of the PFMs showed more profound muscle activation after HIFEM treatments, along with improved relaxation and enhanced endurance. The PFIQ also indicates a greater effect of the HIFEM procedure based on the significant change in patient scores. Documented outcomes imply that the HIFEM procedure is substantially more effective in restoring PFM strength and treating PFD when compared with the electrical stimulation applied correspondingly in postpartum women.

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Statement of Authorship

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REFERENCES

1. Selvan VA. Single-fiber EMG: a review. *Ann Indian Acad Neurol.* 2011; 14: 64-67.
2. Bø K, Sherburn M. Evaluation of female pelvic-floor muscle function and strength. *Phys Ther.* 2005; 85: 269-282.
3. Ribeiro AM, Mateus-Vasconcelos ECL, Silva TD da, et al. Functional assessment of the pelvic floor muscles by electromyography: Is there a normalization in data analysis? A systematic review. F*isioter e Pesqui.* 2018; 25: 88-99.
4. Raez MBI, Hussain MS, Mohd-Yasin F. Techniques of EMG signal analysis: detection, processing, classification, and applications. *Biol Proced Online*. 2006; 8: 11-35.
5. Salsi G, Cataneo I, Dodaro MG, et al. Three-dimensional/four-dimensional transperineal ultrasound: clinical utility and future prospects. *Int J Womens Health.* 2017; 9: 643-656.
6. Braekken IH, Majida M, Engh ME, et al. Test-retest reliability of pelvic floor muscle contraction measured by 4D ultrasound. *Neurourol Urodyn.* 2009; 28: 68-73.
7. García del Salto L, de Miguel Criado J, Aguilera del Hoyo LF, et al. MR imaging-based assessment of the female pelvic floor. *RadioGraphics*. 2014; 34: 1417-1439.
8. Angelo PH, Varella LRD, de Oliveira MCE, et al. A manometry classification to assess pelvic floor muscle function in women. *PLoS One.* 2017; 12.
9. Chamochumbi CCM, Nunes FR, Guirro RRJ, et al. Comparison of active and passive forces of the pelvic floor muscles in women with and without stress urinary incontinence. *Braz J Phys Ther.* 2012; 16: 314-319.
10. Faubion SS, Shuster LT, Bharucha AE. Recognition and management of nonrelaxing pelvic floor dysfunction. *Mayo Clin Proc.*2012; 87: 187-193.
11. Strohbehn K. Normal pelvic floor anatomy. Obstet Gynecol Clin North Am 1998; 25: 683-705.
12. Pemberton J, Swash M, Henry MM, eds. The pelvic floor: its function and disorders. London: Saunders; 2002.
13. Koenig I, Luginbuehl H, Radlinger L. Reliability of pelvic floor muscle electromyography tested on healthy women and women with pelvic floor muscle dysfunction. *Ann Phys Rehabil Med.* 2017; 60: 382-386.
14. Voorham-van der Zalm PJ, Voorham JC, van den Bos TWL, et al. Reliability and differentiation of pelvic floor muscle electromyography measurements in healthy volunteers using a new device: the multiple array probe Leiden (MAPLe). *Neurourol Urodyn*. 2013; 32: 341-348.
15. Zhang Q, Wang L, Zheng W. Surface electromyography of pelvic floor muscles in stress urinary incontinence. *Int J Gynecol Obstet*. 2006; 95: 177-178.
16. Thompson JA, O'Sullivan PB, Briffa NK, et al. Altered muscle activation patterns in symptomatic women during pelvic floor muscle contraction and Valsalva maneuver: altered Muscle Activation Patterns. *Neurourol Urodyn.* 2006; 25: 268-276.
17. Smith MD, Coppieters MW, Hodges PW. Postural response of the pelvic floor and abdominal muscles in women with and without incontinence. *Neurourol Urodyn*. 2007; 26: 377-385.
18. Sapsford RR, Richardson CA, Maher CF, et al. Pelvic floor muscle activity in different sitting postures in the continent and incontinent women. *Arch Phys Med Rehabil.* 2008; 89: 1741-1747.
19. Pereira LC, Botelho S, Marques J, et al. Are transversus abdominis/oblique internal and pelvic floor muscles coactivated during pregnancy and postpartum? *Neurourol Urodyn.* 2013; 32: 416-419.

20. Lauper M, Kuhn A, Gerber R, et al. Pelvic floor stimulation: What are the good vibrations? *Neurourol Urodyn*. 2009; 28: 405-410.
21. Junginger B, Baessler K, Sapsford R, et al. Effect of abdominal and pelvic floor tasks on muscle activity, abdominal pressure, and bladder neck. *Int Urogynecol J.* 2010; 21: 69-77.
22. Gagnon L-H, Boucher J, Robert M. Impact of pelvic floor muscle training in the postpartum period. *Int Urogynecol J.* 2015; 27: 255-260.
23. Zuchelo LTS, Bezerra IMP, Da Silva ATM, et al. Questionnaires to evaluate pelvic floor dysfunction in the postpartum period: a systematic review. *Int J Womens Health.* 2018; 10: 409-424.
24. Radziminska A, Straczynska A, Weber-Rajek M, et al. The impact of pelvic floor muscle training on the quality of life of women with urinary incontinence: a systematic literature review. *Clin Interv Aging.* 2018; 13: 957-965.
25. Correia GN, Pereira VS, Hirakawa HS, et al. Effects of surface and intravaginal electrical stimulation in treating women with stress urinary incontinence: randomized controlled trial. *Eur J Obstet Gynecol Reprod Biol.* 2014; 173: 113-118.
26. Schreiner L, Santos TG dos, Souza ABA de, et al. Electrical stimulation for urinary incontinence in women: a systematic review. *Int Braz J Urol.* 2013; 39: 454-464.
27. Samuels JB, Pezzella A, Berenholz J, et al. Safety and efficacy of a noninvasive high-intensity focused electromagnetic field (HIFEM) device for treating urinary incontinence and enhancing quality of life. *Lasers Surg Med.* 2019; 51: 760-766.
28. Hlavinka T, Turcan P, Bader A. The use of HIFEM technology in the treatment of pelvic floor muscles as a cause of female sexual dysfunction: a multi-center pilot study. *J Womens Health Care.* 2019; 08.
29. Alinsod R, Vasilev V, Yanev K, et al. Hifem technology is a new perspective in the treatment of stress urinary incontinence. *Lasers Surg Med.* 2018; 50
30. Omar A, Marwaha K, Bollu PC. Physiology, neuromuscular junction. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2019; Available at: http://www.ncbi.nlm.nih.gov/books/NBK470413/. Accessed December 5, 2019.
31. Faraday MV. Experimental research in electricity. *Philos Trans R Soc Lond.* 1832; 122: 125-162.
32. Kanjanapanang N, Chang K-V. Peripheral magnetic stimulation (transcutaneous magnetic stimulation). In: *StatPearls*. Treasure Island, FL: StatPearls Publishing; 2019; Available at: http://www.ncbi.nlm.nih.gov/books/NBK526087/. Accessed May 29, 2019.
33. Mehler B, Larsson KM. Electromyographic (EMG) biofeedback in the treatment of pelvic floor disorders. In: Mostofsky DI, ed. The handbook of behavioral medicine. Oxford, UK: John Wiley & Sons, Ltd; 2014; 313-338.
34. Barber MD, Kuchibhatla MN, Pieper CF, et al. Psychometric evaluation of 2 comprehensive condition-specific quality of life instruments for women with pelvic floor disorders. *Am J Obstet Gynecol.* 2001; 185: 1388-1395.
35. De Luca CJ. The use of surface electromyography in biomechanics. *J Appl Biomech.* 1997; 13: 135-163.
36. Coletta NA, Mallette MM, Gabriel DA, et al. Core and skin temperature influences on the surface electromyographic responses to an isometric force and position task. *PLoS One.* 2018; 13
37. Basmajian JV, Luca CJD. Muscles alive: their functions revealed by electromyography. Subsequent edition. Baltimore: Williams & Wilkins; 1985.