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Original Research Article

Evaluating the effects of platelet-rich plasmas plus dehydroepiandrosterone and dehydroepiandrosterone alone in infertile women with diminished ovarian reserve

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ABSTRACT

Background: Diminished ovarian reserve (DOR) is a significant cause of female infertility, often associated with poor response to ovarian stimulation during assisted reproductive techniques. This study aimed to evaluate the effects of a combination therapy using platelet-rich plasma (PRP) and dehydroepiandrosterone (DHEA) compared to DHEA alone in infertile women with DOR.
Methods: This Quasi-experimental study was conducted in the Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from July 2022 to June 2023. In this study, we included 36 women aged 20 to 39 years diagnosed with diminished ovarian reserve (DOR) attending the outpatient Department of Reproductive Endocrinology and Infertility at BSMMU. Participants were assigned to two treatment groups: group A received DHEA plus PRP, and group B received only DHEA.
Results: The mean age in group A was 31.5±5.5 years (range: 25–39), and in group B, it was 31.4±5.2 years (range: 25–39). Baseline characteristics, including age, body mass index (BMI), infertility duration, and hormonal levels, were similar between the groups. Over the 3-month follow-up, both groups showed improvements in ovarian reserve markers. Group A demonstrated slightly greater improvements in AMH (0.36±0.2 ng/ml versus 0.30±0.1 ng/ml), AFC (1.2±1.4 versus 0.82±1.5), and a greater reduction in FSH (-3.1±1.9 mIU/ml versus -2.82±1.7 mIU/ml) compared to group B. However, these differences were not statistically significant.
Conclusions: This study showed that both DHEA alone and in combination with PRP resulted in improved ovarian reserve markers in women with DOR. Although the combination therapy of PRP and DHEA showed slightly better outcomes compared to DHEA alone, the differences were not significant.

Keywords: Diminished ovarian reserve, Platelet-rich plasma, Dehydroepiandrosterone, Infertility

INTRODUCTION

Infertility is a serious problem for world health.[1] Infertility affects approximately 17.5% of the global population, with some regions, such as the Western Pacific, Sub-Saharan Africa, and South East Asia, reporting even higher rates.[2-4] In the United States, 1.5 million women face infertility, with 25% of couples having multiple contributing factors.[5,6] In India, primary infertility prevalence ranges between 3.9% and 16.8%, while in Bangladesh, 4% of couples are affected, particularly women aged 45–49.[5,7,8]

One major contributor to infertility is diminished ovarian reserve (DOR), defined as a decrease in both the quantity and quality of oocytes. It affects approximately 10% of women seeking fertility treatment and is associated with poor assisted reproductive technologies (ART) outcomes.[9,10] Contributing factors include aging, delayed childbearing, and ovarian insufficiency. Diagnostic tests for DOR include antral follicle count (AFC), anti-Müllerian hormone (AMH), basal FSH, estradiol, and inhibin B levels.[11]

Traditional ART treatments for DOR involve high-dose ovarian stimulation, which is costly and often ineffective in poor responders. When ovarian stimulation fails, ovum or embryo donation becomes the only viable option, although not always socially or religiously acceptable, especially in countries like Bangladesh.

Recent research has focused on improving ovarian responsiveness through novel adjuvant therapies such as dehydroepiandrosterone (DHEA) and platelet-rich plasma (PRP). Recently, it has been suggested that androgens play an essential role in folliculogenesis. Dehydroepiandrosterone (DHEA) supplementation is a relatively recent development in the armamentarium for the management of female infertility, used primarily in women with DOR. DHEA has shown promise in enhancing folliculogenesis, increasing AMH levels, reducing aneuploidy, and improving both oocyte quantity and quality.[12] PRP, rich in growth factors, promotes follicular development and ovarian rejuvenation. Studies have demonstrated PRP's potential to improve hormonal profiles and ovarian reserve markers, with early evidence of improved IVF outcomes.[13-15] In a randomized clinical trial, Salih et al reported a significant increase in AMH levels among subfertile Sudanese women following laparoscopic autologous PRP injections.[13] Pantos et al demonstrated that autologous PRP treatment led to ovarian rejuvenation and reactivation of folliculogenesis in peri-menopausal women.[16] Sills et al extended PRP application to ovarian tissue of women with DOR; evidence of improved ovarian function was noted in all as early as two months after treatment.[17]

The combination of DHEA and intra-ovarian PRP represents a promising experimental approach for women with DOR, especially in countries like Bangladesh, where third-party reproduction is not religiously or psycho-socially acceptable. Therefore, in this study, we aimed to evaluate the effectiveness of DHEA alone versus DHEA combined with PRP in enhancing ovarian reserve markers in women undergoing fertility treatment.

METHODS

This Quasi-experimental study was conducted in the Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from July 2022 to June 2023. In this study, we included 36 women aged 20 to 39 years diagnosed with DOR attending the outpatient department of Reproductive Endocrinology and Infertility at BSMMU. Participants were assigned to two treatment groups: group A received DHEA plus PRP, and group B received only DHEA.

These are the following criteria to be eligible for enrollment as our study participants.

Inclusion criteria

Women aged 20-39 years; women with primary or secondary infertility; diminished ovarian reserve with the presence of at least two criteria of the following - serum AMH <1 ng/ml, basal FSH >10 IU/l, and summation of bilateral AFC ≤6 follicles in both ovaries were included.

Exclusion criteria

Women with FSH >25 IU/l and AMH <0.5 ng/ml; women with previous ovarian surgery, and previous chemotherapy or radiotherapy; women with endocrine and/or autoimmune disease (diabetes mellitus, thyroid disorder); women with chronic kidney disease, hepatic dysfunction, and pelvic endometriosis; and women taking supplementation of any drug in the previous 3 months (DHEA, melatonin, vitamin D, co-enzyme Q, and oral contraceptive pill, which could affect the ovarian reserve), were excluded.

Intervention

The study population comprised diagnosed cases of subfertile women with diminished ovarian reserve (DOR). A total of 36 women were selected by purposive sampling according to the inclusion and exclusion criteria and were then divided into two groups. There were two groups of women, group A and group B.

Group A

Group A included patients selected for laparoscopic tube-peritoneal evaluation, who received DHEA orally in a dose of one tablet containing 25 mg three times daily after meals for 12 weeks, along with 5 ml of pre-prepared autologous PRP injected into each ovary during the study period.

Group B

Group B patients received DHEA orally in a dose of one tablet containing 25 mg three times daily after meals for 12 weeks.

Study procedure

Informed written consent was obtained from each participant or their guardians after a full explanation of the study procedure. Serum AMH levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (AMH Gen II ELISA: Beckman Coulter and R & D

Automated Systems). Antral follicle count (AFC) was defined as those measuring 2-10 mm in largest mean diameter on two-dimensional transvaginal ultrasound on days 2-5 of the menstrual cycle, using a cut-off value of 6 follicles in both ovaries. AFC was detected using a GEHCLOGICP7 medical ultrasonography machine with a 7.5 MHz vaginal transducer by the same investigator on days 2-5 of the menstrual cycle. After counseling, obtaining informed written consent, and conducting pre-anesthetic checkups, laparoscopic evaluations under general anesthesia were performed. During laparoscopy, 5 ml of pre-prepared autologous PRP was injected into each ovary.

Autologous PRP preparation

This process began with the insertion of a 21 G butterfly needle into the median cubital vein to obtain 30 ml of fresh whole blood into six CBC tubes containing ethylene diamine tetraacetic acid (EDTA). The white blood cells and platelets were separated from the red blood cells and serum by centrifugation for 10 minutes at 1000-1200 rpm. The bottom layer consisted of RBCs, buffy coats in the middle, and PRP on top. The supernatant top layer was aspirated as PRP and was ready for use. From 30 ml of venous blood, 10 ml of PRP was obtained. The preparation of PRP required less than one hour. Baseline and follow-up evaluations of serum AMH, AFC, and follicle stimulating hormone (FSH) were recorded at baseline, 4 weeks, 8 weeks, and 3 months post-treatment.

Data collection and analysis

Data were collected through interviews, physical and lab examinations using a structured questionnaire containing all variables of interest. All data were recorded systematically in a pre-formatted data collection form. Quantitative data was expressed as mean and standard deviation, and qualitative data was expressed as frequency distribution and percentage. The independent t-test was used to compare symmetrically distributed continuous variables between groups. Chi-square test and Fisher's exact test were used to compare categorical variables. A p value <0.05 was considered significant. Statistical analysis was performed by using statistical package for social sciences (SPSS) 19 for Windows version 26. This study was ethically approved by the Institutional Review Committee of BSMMU, Dhaka, Bangladesh.

RESULTS

This quasi-experimental study was conducted in the Department of Reproductive Endocrinology and Infertility at BSMMU. A total of 36 women, aged between 20 and 39 years and diagnosed with DOR based on ovarian reserve markers (AMH <1 ng/ml), were enrolled. Of these, 18 women received a combination of PRP and DHEA treatment (group A), while the remaining 18 received DHEA alone (group B).

Table 1 shows that in group A, 44.4% were below 30 years, while 55.6% were aged 30 or above. Similarly, in group B, 38.9% were below 30, and 61.1% were 30 or older. The categorical age distribution between the groups was not statistically significant (p=1.00). The mean age in group A was 31.5±5.5 years (range: 25–39), and in group B, it was 31.4±5.2 years (range: 25–39), with no significant difference observed (p=0.777).

Table 1: Distribution of the patients according to age group (n=36).

Age group (years)	Group A (n=18) (%)	Group B (n=18) (%)	P value
<30	8 (44.4)	7 (38.9)	1.00
≥30	10 (55.6)	11 (61.1)	
Mean±SD (range)	31.5±5.5 (39-25)	31.4±5.2 (39-25)	0.777

Group A=DHEA plus PRP, group B=only DHEA

Figure 1 shows that in group A, 38.9% of the females had primary infertility and 61.1% had secondary infertility, as well as in group B, 44.4% of the females had primary and 55.6% had secondary infertility.

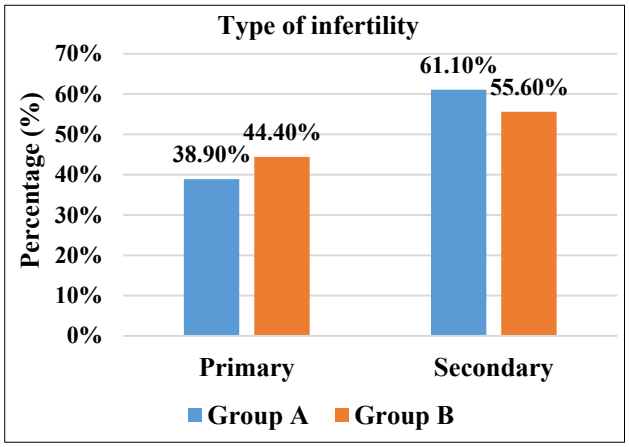

Figure 1: Distribution of the women according to the type of infertility (n=36).

Group A=DHEA plus PRP, group B=only DHEA

Table 2 shows that in both group A and group B, the majority had irregular menstrual cycles (66.7% versus 72.2%). The mean BMI of the females was 22.3±1.6 kg/m² and 22.5±1.6 kg/m² in group A and group B, respectively. The mean age of menarche was 13.2±1 years, and the duration of infertility was 3.5±0.6 years in group A, while in group B, the mean age of menarche was 13±1 years and the duration of infertility was 3.6±0.6 years.

Table 3 shows that before treatment, the mean serum AMH was 0.77±0.1 ng/ml, AFC was 4.2±1, and serum FSH was 12.5±1.5 mIU/ml in group A. Additionally, in group B, the values were 0.77±0.1 ng/ml, 4.3±1.1, and 12.5±1.6 mIU/ml, respectively. No significant difference was found between the two groups.

Table 4 shows that after 4 weeks of treatment, mean serum AMH, AFC, and serum FSH were 0.86±0.1 ng/ml, 4.5±0.9, and 11.9±1.7 mIU/ml in group A and 0.85±0.1 ng/ml, 4.4±0.9, and 12±1.6 mIU/ml in group B, respectively. No significant difference was found between the groups.

Table 2: Baseline characteristics of the study participants (n=36).

Menstrual cycle	Group A (n=18), (%)	Group B (n=18), (%)	P value
Regular	6 (33.3)	5 (27.8)	1.00^{ns}
Irregular	12 (66.7)	13 (72.2)	
Age of menarche (year)	13.2±1	13±1	0.739^{ns}
BMI (kg/m^2)	22.3±1.6	22.5±1.6	0.647^{ns}
Duration of infertility	3.5±0.6	3.6±0.6	0.789^{ns}

Group A=DHEA plus PRP, group B=only DHEA; ns=not-significant

Table 3: Study-related baseline investigations of the participants (n=36).

Variables	Group A (n=18) (mean±SD)	Group B (n=18) (mean±SD)	P value
Serum AMH (ng/ml)	0.77±0.1	0.78±0.1	0.769
AFC	4.2±1	4.3±1.1	0.748
Serum FSH (mIU/ml)	12.5±1.5	12.5±1.6	0.838

Group A=DHEA plus PRP, group B=only DHEA, AMH=anti-Müllerian hormone, AFC=antral follicle count, FSH=follicle-stimulating hormone

Table 4: Post-treatment assessment of ovarian reserve markers after 4 weeks among the infertile women of both groups (n=34).

Ovarian reserve markers	Group A (n=17)* (mean±SD)	Group B (n=17)* (mean±SD)	P value
Serum AMH (ng/ml)	0.86±0.1	0.85±0.1	0.075^{ns}
AFC	4.5±0.9	4.4±0.9	0.851^{ns}
Serum FSH (mIU/ml)	11.9±1.7	12±1.6	0.839^{ns}

ns=Not significant, group A=DHEA plus PRP, group B=only DHEA, *1 patient was dropped during follow up

Table 5 shows that after 8 weeks of treatment, mean serum AMH, AFC, and serum FSH were 1±0.1 ng/ml, 5.2±0.9, and 9.4±1.3 mIU/ml in group A and 1±0.1 ng/ml, 4.9±1.1, and 10±1.6 mIU/ml in group B, respectively. No significant difference was found between the groups.

Table 6 shows that after 3 months of treatment, serum AMH, AFC, and serum FSH were 1.14±0.2 ng/ml, 5.4±0.9, and 9.3±1.3 mIU/ml in group A and 1.08±0.1 ng/ml, 5.1±0.1, and 9.7±1.7 mIU/ml in group B, respectively. No significant difference was found between the groups.

Table 5: Post-treatment assessment of ovarian reserve markers after 8 weeks among the infertile women of both groups (n=34).

Ovarian reserve markers	Group A (n=17)* (mean±SD)	Group B (n=17)* (mean±SD)	P value
Serum AMH (ng/ml)	1±0.1	1±0.1	0.600^{ns}
AFC	5.2±0.9	4.9±1.1	0.413^{ns}
Serum FSH (mIU/ml)	9.4±1.3	10±1.6	0.238^{ns}

ns=Not significant, group A=DHEA plus PRP, group B=only DHEA, *1 patient was dropped during follow up

Table 6: Post-treatment assessment of ovarian reserve markers among the infertile women of both groups after 12 weeks (n=34).

Ovarian reserve markers	Group A (n=17)* (mean±SD)	Group B (n=17)* (mean±SD)	P value
Serum AMH (ng/ml)	1.14±0.2	1.08±0.1	0.327^{ns}
AFC	5.4±0.9	5.1±0.1	0.288^{ns}
Serum FSH (mIU/ml)	9.3±1.3	9.7±1.7	0.509^{ns}

ns=Not significant, group A=DHEA plus PRP, group B=only DHEA, *1 patient was dropped during follow up

Table 7 shows that after 3 months of treatment, mean differences of serum AMH, AFC, and serum FSH were 0.36±0.2 ng/ml, 1.2±1.4, and -3.1±1.9 mIU/mL in group A and 0.30±0.1 ng/ml, 0.82±1.5, and -2.82±1.7 mIU/ml in group B, respectively. Improvement was higher in the combination group, but no significant difference was found between the groups.

Table 7: Comparison of mean difference of post-treatment ovarian reserve markers from baseline among the infertile women (n=34).

Ovarian reserve markers	Group A (n=17) (mean±SD)	Group B (n=17) (mean±SD)	P value
Serum AMH (ng/ml)	0.36±0.2	0.30±0.1	0.284^{ns}
AFC	1.2±1.4	0.82±1.5	0.408^{ns}
Serum FSH (mIU/ml)	-3.1±1.9	-2.82±2.3	0.602^{ns}

ns=Not significant, group A=DHEA plus PRP, group B=only DHEA

DISCUSSION

This current study aimed to evaluate and compare the effects of PRP plus DHEA with DHEA alone on ovarian reserve markers in infertile women with diminished ovarian reserve (DOR). A total of 36 women were enrolled, with 18 receiving PRP plus DHEA (group A) and 18 receiving DHEA alone (group B). During the 3-month follow-up period, one participant from each group was lost to follow-up, and outcomes were analyzed in 17 women per group.

In this study, in both group A and group B, the majority of the female participants were 30 years or above (55.6% versus 61.1%), with corresponding mean ages of 31.5±5.5 and 31.4±5.2 years. A previous study was conducted among infertile women, whereas the mean age was 33.28±3.13 years and 34.16±4.27 years in the DHEA group and control group, respectively (p=0.194).[18] Another study was performed to assess the efficacy of autologous PRP, whereas the median age was 41 years and ranged between 39 to 44 years in both the PRP group and the control group (p=0.78).[19] Uddin et al found the mean age of the infertile women was 35.9±3.2 years.[20] Banu et al also observed a similar age distribution with 36.4 years among the infertile women.[21] The most common age range for women experiencing infertility issues is in their mid-30s to early 40s. This is because fertility starts to decline around age 30 and becomes more significant after age 35, with a marked decrease in the number and quality of eggs. Consequently, women in this age range often face greater challenges in conceiving naturally.

Whereas 38.9% of the females in group A had primary infertility and 61.1% had secondary infertility, group B comprised 44.4% of the females with primary infertility and 55.6% with secondary infertility. This was consistent with the previous study observed that more than fifty-five percent of the patients had the secondary type of infertility.[18] But in another study, primary infertility was found among 90 (81%), and secondary infertility among 21 (18.9%).[5] Both primary and secondary infertility are prevalent in the 20 to 39 age group, with secondary infertility becoming more common as women get older and after they have had one or more pregnancies.[22]

Among all the females in both group A and group B majority had irregular menstruation cycles (66.7% versus 72.2%). A previous study also declared that the most frequent causes of female infertility are uterine factors, menstrual and ovulation disorders, and ovarian disorders.[23]

Mean serum AMH, AFC, and FSH before therapy were 0.77±0.1 ng/ml, 4.2±1, and 12.5±1.5 mIU/ml, respectively, in group A; besides, 0.77±0.1 ng/ml, 4.3±1.1, and 12.5±1.6 mIU/ml, respectively, in group B. One female participant in each group was removed from the trial after three months of treatment because she had not returned for follow-up. After 12th week of treatment, serum AMH, AFC and serum FSH was 1.14±0.2 ng/ml, 5.4±0.9 and 9.3±1.3 mIU/ml in group A and 1.08±0.1 ng/ml, 5.1±0.1 and 9.7±1.7 mIU/ml in group B. Mean serum FSH level were significantly decreased and serum AMH level and AFC were significantly increased after treatment in both group A and group B. Mean difference was found slightly higher in group A than group B which represents that combination group showed higher improvement than single group but no significant difference was found between both groups.

Prior research suggested that PRP might be considered a useful rejuvenation technique because it had a significant favorable effect on infertile women.[13] A study by Melo et al suggested that PRP injections can safely and effectively improve the markers of poor ovarian reserve.[19] Uddin et al also considered injecting autologous PRP into human ovaries as a safe procedure to improve ovarian reserve markers.[20] Another quasi-experimental study conducted by Banu et al revealed significant improvement in AMH and AFC values following PRP infusion.[21] Another study found that DHEA treatment can increase AR expression in preovulatory GCs both *in vitro* and *in vivo*. The favorable effects of DHEA supplementation on ovarian responsiveness in DOR women may be selectively attributed to the enhanced expression of AR and FSHR in GCs.[18] An earlier study involved splitting infertile women into two groups: those taking only DHEA and those taking DHEA plus vitamin D. However, the combination group outperformed the only DHEA group in terms of efficacy.[24] A study done by Barad et al revealed no significant improvement after PRP among infertile women.[25]

In the current study, aged group below 30 years showed higher improvement than 30 and above years aged group during the follow-up in both the single and combination groups. Similar findings were also revealed by a previous study done by Melo et al, which suggested that PRP may represent a more effective treatment for infertility in younger women than older women.[19] Another previous study also concluded that the combination of PRP and DHEA appears to offer a more effective treatment for infertile women with diminished ovarian reserve compared to DHEA alone, with younger women generally experiencing more pronounced benefits.[25]

Limitations

Our study was a single-center study, and the study period was short. We took a small sample size, so it does not represent the whole community. After evaluating those patients, we did not follow up with them for the long term and did not know other possible interference that may happen in the long term with these patients.

CONCLUSION

In our study, we found that both DHEA alone and the combination of DHEA with PRP significantly improved ovarian reserve markers in women with diminished ovarian reserve over three months. Both treatment groups

experienced reductions in serum FSH levels and increases in serum AMH levels and AFC. No significant differences were observed between the two groups post-treatment, indicating that both treatment approaches were equally effective.

Further study with a prospective and longitudinal study design, including a larger sample size with long-term follow-up, needs to be done to validate the efficacy and safety of DHEA and PRP supplementation in improving ovarian reserve and fertility outcomes.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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